IOBC / WPRS
Working group „Insect Pathogens and Insect Parasitic Nematodes”
Subgroup “Soil Insect Pests”

OILB / SROP
Groupe du travail “Entomopathogènes et Nématodes Parasites
d’Insectes”
Sous-Groupe “ Soil Insect Pests ”

PROCEEDINGS of the MEETING
COMPTES RENDUS de la REUNION

at / à

Auer/Ora (Italy)
16-18 October 2006

Editor:
Jürg Enkerli

IOBC wprs Bulletin
Bulletin OILB srop
Vol. 30(7) 2007
Preface

This bulletin contains the proceedings of the 5th meeting of the IOBC/WPRS working group “Insect Pathogens and Insect Parasitic Nematodes” subgroup “Soil Insect Pests”, previously subgroup “Melolontha”, held in Auer/Ora, Italy, 16-18 October 2006.

At the last meeting in October 2004 in Innsbruck, Austria, the subgroup “Melolontha” decided to broaden its focus from strictly “Melolontha” to other soil dwelling pests and as a consequence the subgroup “Melolontha” was renamed to “Soil Insect Pests”. The subgroup in the future will focus on topics like “Scarabaeidae” and wireworms but will also include other emerging soil dwelling pests of interest.

Forty-four scientists predominately from Europe attended the meeting in Auer/Ora, which offered a very interesting program consisting of 28 oral and 12 poster contributions. Five invited presentation reviewed the commercial use of biological control, biosafety aspects and regulations of bacterial and fungal biocontrol agents, experiences with cockchafer control in the South Tyrol, perspectives and possibilities of wireworm control, and biology of Hyalesthes obsoletus Signoret and approaches to control this soilborne vector of grapevine Bois noir disease. The 23 offered presentations and the posters reported on various aspects of detection, monitoring and biological control of “Scarabaeidae” and “wireworms” as well as some other soil insect pests like e.g. weevils and maggots.

On behalf of the subgroup and all attendants I would like express my gratitude to the local organizers Dr. Wolfgang Schweigkofler and Dr. Roland Zelger both from the Research Centre for Agriculture and Forestry, Laimburg, Italy for organizing this very interesting and fruitful meeting. Many sponsors supported the working group meeting and contributed substantially to its success. They are greatly acknowledged.

After the last meeting in October 2004 and after 10 years of successfully leading the subgroup, Siegfried Keller has decided to resign as convenor and to pass on the duties. In the name of the IOBC subgroup “Soil Insect Pests” I wish to thank him for all the energy and time he has put into this group and into the organization of the numerous subgroup meetings. His activities allowed all of us to benefit in many ways on a scientific as well as a personal level.

Jürg Enkerli
Convenor of the subgroup
List of Participants

BENKER Ullrich  
Bavarian State Research Centre for Agriculture (LfL)  
Institute for Plant Protection  
Lange Point 10  
D-85354 Freising  
Germany  
Tel.: 0049 8161 715720,  
Fax: 0049 8161 715753,  
E-mail: Ullrich.Benker@LfL.bayern.de

BLACKSHAW Rod P.  
School of Biological Sciences  
University of Plymouth  
Drake Circus  
Plymouth PL4 8AA  
UK  
Tel.: 0044 780 3008302  
Fax: 0044 175 2232970  
E-mail: rblackshaw@plymouth.ac.uk

BRUNNER Nina  
Bio Forschung Austria  
Rinnböckstraße 15  
1110 Wien  
Austria  
Tel.: 0043 1 7129899  
Fax: 0043 1 795149997940  
E-mail: n.brunner@bioforschung.at

EHLERS Ralf-Udo  
Institute of Phytopathology  
University Kiel  
Hermann-Rodewald Str. 9  
24118 Kiel  
Germany  
E-mail: Ehlers@biotec.uni-kiel.de

ENKERLI Jürg  
Agroscope Reckenholz-Tänikon  
Research Station ART  
Reckenholzstr. 191  
8046 Zurich  
Switzerland  
Tel.: 0041 44 3777206  
Fax: 0041 44 3777201  
E-mail: juerg.enkerli@art.admin.ch

ESTER Albert  
Applied Plant Research (PPO-AGV)  
Wageningen University and Research  
P.O.Box 430  
8200 AK Lelystad  
The Netherlands  
E-Mail: Albert.Ester@wur.nl

FURLAN Lorenzo  
Department of Agronomy  
Entomology, University of Padova  
Agripolis, via Romea 16  
Legnaro PD  
Italy  
E-mail: lorenzo.furlan@inwind.it

GHORMADE Vandana  
Agroscope Reckenholz-Tänikon  
Research Station ART  
Reckenholzstrasse 191  
CH-8046 Zürich  
Switzerland  
Tel.: 0041 44 3777593  
Fax: 0041 44 3777201  
E-mail: vandana.ghormade@art.admin.ch

HICKS Helen  
Terrestrial ecology research group  
University of Plymouth  
Drake Circus  
Plymouth, PL4 8AA  
UK  
Tel.: 0044 780 3008302  
Fax: 0044 175 2232970  
E-mail: helen.hicks@plymouth.ac.uk

HUTWIMMER Stefan  
Institute of Microbiology  
LFU Innsbruck  
Technikerstr. 25  
6020 Innsbruck  
Tel. 0043 512 5076015  
Fax: 0043 512 5072929  
E-mail: stefan.hutwimmer@uibk.ac.at
JARONSKI Stefan T.
USDA ARS Northern Plains Agricultural Research Lab
1500 N. Central Ave.
Sidney MT 5927
Tel.: 001 406 4339486
Fax: 001 206 4335038
E-mail: sjaronski@sidney.ars.usda.gov

KIRCHMAIR Martin
Institute of Microbiology
LFU Innsbruck
Technikerstr. 25
6020 Innsbruck
Tel.: 0043 512 5076013
Fax: 0043 512 5072938
E-mail: martin.kirchmair@uibk.ac.at

JUNG Kerstin
Federal Biological Research Centre for Agriculture and Forestry
Institute for Biological Control
Heinrichstr. 243
64287 Darmstadt
Germany
E-mail: k.jung@bba.de

KROMP Bernhard
Bio Forschung Austria
Rinnböckstr. 15
1110 Vienna
Austria
Tel.: 0043 1 712 9899
Fax: 0043 1 7951499 997940
E-mail: b.kromp@bioforschung.at

KABALUK Todd
Agriculture and Agri-Food Canada
c/o Box 1000
Agassiz
British Columbia VOM 1A0
Canada
Tel.: 001 604 7962221
Fax: 001 604 7960359
E-mail: kabalukt@agr.gc.ca

KRON MORELLI Roberto
Agrifutur SRL
Via Campagnole 8
25020 Alfianello
Italy
Tel. 0039 30 9934776
Fax: 0039 30 9934777
E-mail: rkm@numerica.it

KATZUR Katrin
BBA, Institute for Plant Protection in Field Crops and Grassland
Messeweg 11-12
D-38104 Braunschweig
Germany
Tel.: 0049 531 2994576
Fax: 0049 531 2993008
E-mail: K.Katzur@bba.de

LÖSCH Angelika
Institute of Microbiology
LFU Innsbruck
Technikerstr. 25
6020 Innsbruck
Tel.: 0043 512 5076015
Fax: 0043 512 5072929

MAIXNER Michael
Biologische Bundesanstalt für Land- und Forstwirtschaft (BBA)
Brüningstr. 84
54470 Bernkastel-Kues
Germany
Tel.: 0049 6531 971821
Fax: 0049 6531 4936
E-mail: m.maixner@bba.de

KELLER Siegfried
Agroscope Reckenholz-Tänikon
Research Station ART
Reckenholzstrasse 191
CH-8046 Zurich
Switzerland
Tel.: 0041 44 377721
Fax: 0041 44 3777201
E-mail: Siegfried.keller@art.admin.ch
MIKKELSEN Harald
Koppert Biological Systems
Veilingweg 17
2651 BE Berkel en Rodenrijs
The Netherlands
Tel.: 0021 10 5140444
Fax: 0031 10 5121005
E-mail: hmiikkelsen@koppert.nl

MURRAY Phil
Cross Institute Programme for Sustainable Soil Function, Institute of Grassland and Environmental Research
North Wyke Research Station
Okehampton
Devon, EX20 2SB
UK
Fax: 0044 183 782139
E-mail: phil.murray@bbsrc.ac.uk

NEUHOFF Daniel
Institute of Organic Agriculture
University of Bonn
Katzenburgweg 3
53111 Bonn
Germany
Tel.: 0049 228 732883
Fax: 0049 228 735617
E-mail: d.neuhoff@uni-bonn.de

OSTRAUSKAS Henrikas
Phytosanitary Research Laboratory
State Plant Protection Service
Pelesos str. 85
Vilnius LT11351
Lithuania
Tel.: 00370 5 2616801
Fax: 00370 5 2616801
E-mail: vaathe@vaat.lt

OTT Andreas
Forest Research Institute of Baden-Württemberg
Department of Forest Protection
Wonnhaldestr. 4
79100 Freiburg
Germany
Tel.: 0049 761 4018226
Fax: 0049 761 4018333
E-mail: andreas.ott@forst.bwl.de

PARKER William E.
ADAS, Woodthorne
Wergs Road
Woverhampton, WV6 8TQ
UK
Tel.: 0044 1746 712815
Fax: 0044 1902 748929
E-mail: bill.parker@adas.co.uk

PERNFUSS Barbara
Institute of Microbiology
LFU Innsbruck
Technikerstr. 25
6020 Innsbruck
Tel.: 0043 512 5076012
Fax: 0043 512 5072929
E-mail: Barbara.Pernfuss@uibk.ac.at

PILZ Christina
ART Agroscope Reckenholz-Tänikon
Reckenholzstr. 191
8046-Zürich
Tel.: 0041 13777372
E-mail: pilzchristina@hotmail.com

POZENEL Anka
Institute of Agriculture
Pri hrastu 18
SI-5000 Nova Gorica
Slovenia
Tel.: 00386 5 3671072
Fax: 00386 5 3671073
E-mail: anka.pozenel@go.kgzs.si

PŮŽA Vladimir
Institute of Entomology
Biological Centre
CAS, Branisovska 31
České Budějovice
Czech Republic
Tel.: 0042 038 7775237
E-mail: vladap@bf.jcu.cz

ROT Mojca
Institute of Agriculture
Pri hrastu 18
SI-5000 Nova Gorica
Slovenia
Tel.: 00386 5 3671072
Fax: 00386 5 27312
E-mail: mojca.rot@kvz-ng.si
SCHALLHART Nikolaus
Institute of Ecology
Mountain Agriculture Research Unit
University of Innsbruck
Technikerstrasse 25
6020 Innsbruck
Austria
Tel.: 0043 512 5075689
E-mail: Klaus.Schallhart@uibk.ac.at

SCHWEIGKOFLER Wolfgang
Dept. Plant Protection
Research Centre for Agriculture and Forestry Laimburg
39040 Auer/Ora
Italy
Tel.: 0039 471 969630
Fax: 0039 0471 969579
E-mail: wolfgang.schweigkofler@provinz.bz.it

STANELIS Andrius
Station Plant Protection Service
Pelesos str. 85
11351 Vilnius
Lithuania
Tel.: 00370 5 2616801
Fax: 00370 5 2616801
E-mail: vaatar@vaat.lt

STRASSER Hermann
Institute of Microbiology
LFU Innsbruck
Technikerstr. 25
6020 Innsbruck
E-mail: Hermann.Strasser@uibk.ac.at

SUFYAN Muhammad
Institute of Organic Agriculture
University of Bonn
Katzenburgweg 3
53111 Bonn
Germany
Tel.: 0049 228 732039
E-mail: msufian@uni-bonn.de

TÓTH Miklós
Plant Protection Institute
Hungarian Academy of Science
Budapest, POB 102, H-1525
Hungary
Tel.: 0036 1 3918639
Fax: 0036 1 3918655
E-mail: h2371tot@ella.hu

TRAUGOTT Michael
Institute of Ecology
Mountain Agriculture Research Unit
University of Innsbruck
Technikerstrasse 25
6020 Innsbruck
Austria
Tel.: 0043 512 5075693
E-mail: Michael.Traugott@uibk.ac.at

VERDUN Cyrille
Becker Underwood Ltd
Harwood Road
BN177AU Littlehampton
UK
Tel.: 0044 779 287878
Fax: 0044 1903 732266
E-mail: cyrille.verdun@beckerunderwood.com

VERNON Robert S.
Agriculture and Agri-Food Canada
Pacific Agri-Food Research Centre
PO Box 1000
Agassiz, British Columbia
Canada V0M 1A0
Tel.: 001 604 7962221
Fax: 001 604 7960359
E-mail: vernonbs@agr.gc.ca

VINOTTI Valerio
Agrifutur SRL
Via Campagnole 8
25020 Alfianello
Italy
VUTS Joszef
Plant Prot. Inst. Has
Herman O. u. 15
1022 Budapest
Hungary
Tel.: 0036 1 3918637
Fax: 0036 1 3918655
E-mail: joci2@freemail.hu

WEBER Frans
Koppert Biological Systems
Veilingweg 17
2651 BE Berkel en Rodenrijs
The Netherlands
Tel.: 0031 10 5140444
Fax: 0031 10 5121005
E-mail: fweber@koppert.nl

ZELGER Roland
Dept. Plant Protection
Research Centre for Agriculture and
Forestry Laimburg
39040 Auer/Ora
Italy
Tel.: 0039 471 969633
Fax: 0039 0471 969579
E-mail: roland.zelger@provinz.bz.it
Contents

Preface...........................................................................................................................................iii

List of participants........................................................................................................................v

Contents.........................................................................................................................................xi

Invited Papers

Biology of *Hyalesthes obsoletus* and approaches to control this soilborne vector of Bois noir disease
*M. Maixner*............................................................................................................................. 3

Management and biological control of wireworm populations in Europe: current possibilities and future perspectives
*Lorenzo Furlan*....................................................................................................................... 11

Wireworms

Occurrence of click beetle pest spp. (Coleoptera, Elateridae) in Europe as detected by pheromone traps: survey results of 1998-2006
*Lorenzo Furlan, Miklos Toth, Cooperators*............................................................................... 19

Spatial distribution of click-beetles (*Agriotes* spp.) at field and landscape scales
*Rod P. Blackshaw, Robert S. Vernon, Helen Hicks*................................................................. 27

European wireworms (*Agriotes* spp.) in North America: toxicity and repellency of novel insecticides in the laboratory and field
*Robert S. Vernon, Wim Van Herk, Chandra Moffat, Chantelle Harding*................................. 35

New approaches to wireworm management in the UK
*William E Parker*................................................................................................................... 43

Comparison of three different bait trap types for wireworms (Coleoptera: Elateridae) in arable crops
*Nina Brunner, Eva-Maria Grünbacher, Bernhard Kromp*......................................................... 47

Practical Dutch experience introducing a monitoring system of click beetles by pheromone traps.
*Klaas van Rozen, Albert Ester, Ton Hendrickx*......................................................................... 53

New sex attractant for *Agriotes proximus*: similarities in pheromonal communication with *A. lineatus* (Coleoptera: Elateridae)
*Miklós Tóth, Lorenzo Furlan, Amália Xavier, József Vuts, Mitko Subchev, Teodora Toshova, István Szarukán, Venyamin Yatsynin*....................................................... 59
Approaches to wireworm control in organic potato production
Daniel Neuhoff, Christiana Christen, Andreas Paffrath, Ute Schepl

Promise versus performance: working toward the use of Metarhizium anisopliae as a biological control for wireworms
Todd Kabaluk, Mark Goettel, Jerry Ericsson, Martin Erlandsson, Ffion Cassidy, Bob Vernon, Stefan Jaronski, Kenna Mackenzie, Lee Cosgrove

Evaluation of Metarhizium anisopliae isolates for biocontrol of Agriotes based on genetic, biochemical and virulence characters
Vandana Ghormade, Werner Jossi, Santosh Chavan, Arumugam Rajendran, Amey Ghondhelekar, Franco Widmer, Siegfried Keller, Jürg Enkerli

Investigations on click beetles using pheromone traps
Muhammad Sufyan, Daniel Neuhoff, Lorenzo Furlan

Melolontha and other Scarabaeidae

The swarming flight of Common cockchafer Melolontha melolontha L., 1758 (Coleoptera, Scarabaeidae) in two different areas of Bavaria and an approach to control the egg deposition
Ullrich Benker, Bernhard, Leuprecht

Spraying adult forest cockchafers with Beauveria brongniartii-conidiospores: Preliminary results of a large field trial nearby Darmstadt during the main flight in 2006
Kerstin Jung

White grub control in golf courses
Siegfried Keller, Christian Schweizer

Entomopathogenic nematodes and target soil insect pests in tree habitats in the Czech Republic, with focus on sawflies and cockchafers
Vladimír Půža, Zdeněk Mráček, Jaroslav Holuša

A great increase of population of Common Cockhafer (Melolontha melolontha L.) in Idrija region in Slovenia
Anka Poženel, Mojca Rot

Miscellaneous

Tipula paludosa population dynamics: challenging the myth of environmental limitation
Rod P. Blackshaw, Sergei V. Petrovskii

Challenges in Using Metarhizium anisopliae for Biocontrol of Sugarbeet Root Maggot, Tetanops myopaeformis
Stefan T. Jaronski, Cynthia Fuller-Schaeffer, Kerstin Jung, Ayanava Majumdar, Mark Boetel
Aggregation attractants for the sugar-beet weevils *Bothynodera punctiventris* and *Conorrhynchus mendicus* (Coleoptera, Curculionidae, Cleoninae): application opportunities
*Miklós Tóth, Lorenzo Furlan, Giovanni Campagna, Zoltán Imrei, Ivan Sivcev, Ivan Tomasev, István Ujváry*……………………………………………………………………………… 125

*Metarhizium* spp. against locusts and grasshoppers – a short review and future prospects
*Barbara Pernfuss, Roberto Kron Morelli, Roland Zelger, Hermann Strasser*…. 133

Persistence of GRANMet®, a *Metarhizium anisopliae* based product, in grape phylloxera-infested vineyards
*Martin Kirchmair, Marc Hoffmann, Sigrid Neuhauser, Hermann Strasser, Lars Huber* …………………………………………………………………………………………….. 137

Are genetic algorithms a “magic bullet” for optimising cultivation conditions for entomopathogenic fungi?
*Stefan Hutwimmer, Wolfgang Burgstaller, Hermann Strasser*……………… 143

Assessment of virulence test-systems for quality assurance using sub-cultivated *Beauveria brongniartii* conidia
*Angelika Loesch, Stefan Hutwimmer, Barbara Pernfuss, Hermann Strasser*…. 149
Invited Papers
Biology of *Hyalesthes obsoletus* and approaches to control this soilborne vector of Bois noir disease

M. Maixner  
*BBA, Institute for Plant Protection in Viticulture, D-54470 Bernkastel-Kues, Germany - M.Maixner@BBA.de*

**Abstract:** The planthopper *Hyalesthes obsoletus* is the vector of Bois noir disease of grapevine and diseases of other plants associated with stolbur phytoplasma. The polyphagous vector acquires the pathogen while feeding on the roots of herbaceous reservoir plants. Occasional feeding of adults on grapevine causes infection and subsequent development of Bois noir symptoms. *H. obsoletus* is a xerothermic species that prefers sparse vegetation on open soil. The herbaceous host plants considerably influence distribution and density of *H. obsoletus* as well as the levels of infestation of vector populations. Since specific strains of the Bois noir phytoplasma occur in different host plants and the vector populations are adapted to their particular hosts, too, there are different epidemic cycles of the disease in the field. Control strategies for *H. obsoletus* focus on cultural practices like soil cultivation, weed control and green cover.

**Key words:** *Hyalesthes obsoletus*, Bois noir, epidemiology, control

**Introduction**

Bois noir (BN) is a grapevine yellows disease that is associated to phytoplasmas of the stolbur (16SrXII) group (Daire et al., 1993; Maixner et al., 1994). Though the pathogen is endemic to Europe and widespread in various wild and cultivated plants, BN is still emerging in many European and Mediterranean viticultural regions, where sudden and severe outbreaks occurred recently (Maixner, 2006). The Cixiid planthopper *Hyalesthes obsoletus* Signoret (Hemiptera: Cixiidae) is the only vector known to transmit BN-phytoplasma to grapevine in the field (Maixner et al., 1995; Sforza et al., 1998; Alma et al., 2002). Knowledge of its biology and behaviour and the range of its host plants is a prerequisite for the understanding of BN epidemiology and the development of well-adjusted control strategies. A summary of the current knowledge of the biology of *H. obsoletus* and the approaches to control this vector is presented in this paper.

**Biology of *Hyalesthes obsoletus***

Different authors (Leclant, 1969; Alma et al., 1987; Sforza et al., 1999; Sharon et al., 2005) have studied the life cycle of *H. obsoletus*. It is univoltinious in Europe while two generations appear in Israel (Klein et al., 2001). Eggs are deposited in batches in the upper level of the soil close to the root collar of herbaceous plants. All of the five larval instars feed on the roots of their host plants. Second and third instar larvae hibernate in a depth of 20 to 25 cm (Langer et al., 2003) where they are usually protected from frost damage. Whether they feed during winter is not known. During April and May they gradually move up to just below the soil level where they are often assembled in groups of two to five individuals in cavities that are lined with wax fibers produced by abdominal glands. At the beginning of the flight activity of the adult planthoppers, both fifth instar nymphs and newly molded adults can be found
together in such nests. The speed of development and thereby the start of the flight activity of adult vectors depend on spring temperatures (Maixner & Langer, 2006), but also on the species of host plants (Fig. 1). Populations living on *Convolvulus arvensis* start approximately three to four weeks in advance of those that develop on *Urtica dioica*. A peak activity is reached within the first three weeks. The beginning of the flight activity can be predicted by a degree-day method (Maixner & Langer, 2006); however, it is limited so far to populations living on *C. arvensis*. Most adult *H. obsoletus* remain on the plant stands where they hatched and their immediate surroundings, however, a few individuals could be caught in the middle of a tilled field on traps exposed in heights up to 3.5 m in a distance of at least 50 m from the next host plants. While the sex ratio of *H. obsoletus* is balanced on traps at the soil level close to the host plants, the predominance of male planthoppers on more distant traps hints on a higher flight activity of this sex.

Adult *H. obsoletus* are polyphagous and feed on a wide range of herbaceous plant species. Grapevine, however, is only an erroneous feeding host (Vidano et al., 1988; Sforza & Boudon-Padieu, 1998). The immature stages are restricted to fewer host plant species. Most important in Europe are field bindweed (*Convolvulus arvensis*), stinging nettle (*Urtica dioica*), perennial species of *Ranunculus*, and hedge bindweed (*Calystegia sepium*). In Israel they live also on the shrub *Vitex agnus-castus* (Sharon et al., 2005). The populations of *H. obsoletus* living on *C. arvensis* or on *U. dioica* are considerably separated because of the delayed flight activity of the insects on nettle. Significantly different survival rates of the planthoppers on their homologous compared to their heterologous host plants hint to a further adaptation of the vector to different host plants (Fig. 2).

There are regional differences with respect to the host plants that are preferred by *H. obsoletus*. This species was more or less restricted to *C. arvensis* in Germany and probably also in other northern viticultural regions, while *U. dioica* is the preferred host plant e.g. in northern Italy (Conti & Vidano, 1988; Credi, 2006). Only about five years ago *H. obsoletus* started to exploit *U. dioica* as a host plant in Germany, too, and it is now widely distributed on this species. Environmental conditions, namely rising temperatures are suspected to be

![Graph](image-url)

**Figure 1**: Flight periods of host specific populations of *H. obsoletus*. Sweep net catches on *C. arvensis* and *U. dioica* in a vineyard of the Middle-Rhine area in 2005. Stands of host plants were swept weekly until no further planthoppers were caught.
responsible for this phenomenon. *H. obsoletus* reaches the northern border of its range in Germany, where it is restricted to areas with favourable microclimate such as vineyards. Typical sites are characterised by xerothemic conditions. Loose and stony soils are favourable since they provide the crevices required by the immature planthoppers to move to their hibernation sites.

*H. obsoletus* larvae exhibit a highly aggregated spatial distribution. Even though it is clearly linked to the dispersion of the host plants, numbers of planthoppers vary considerably from plant to plant. Less vigorous plants growing sparsely on otherwise open soil are

![Figure 2: Longevity of adult *H. obsoletus* collected from *C. arvensis* and *U. dioica*, respectively, in the field and kept in cages on either the homologous or heterologous host species (means ± standard error).](image)

![Figure 3: Comparison of trap catches (horizontal sticky traps on the soil) of *H. obsoletus* on green covered and open soil (means ± standard error).](image)
preferred compared to dense stands of host plants or to green covered soil (Fig. 3).

**Role of *Hyalesthes obsoletus* for the epidemiology of Bois noir**

The BN-phytoplasma is maintained in the field by a cyclic host change between herbaceous reservoir plants and the vector *H. obsoletus*. Since the immature vectors already acquire the pathogens from their hosts, the adult planthoppers are infective when they emerge from the soil. There is no significant increase of infestation levels in the vector populations during the flight period, which indicates that acquisition of phytoplasmas by the adult vectors is of minor significance (Darimont & Maixner, 2001). Acquisition of BN-phytoplasma from grapevine is unlikely, due to the feeding preferences of the vector. Grapevine is therefore considered to be a dead end host for the phytoplasma without any role in the epidemiology of the disease.

The infestation levels of the vector populations depend on the predominant host plants. High proportions of infected vectors between 30 to 80 % are typical for populations on *C. arvensis* while less than 5 % infected insects are usually found where *Ranunculus* species are the predominant hosts. This difference is due to the differential behavior of the infected plants. Infected *C. arvensis* plants - though exhibiting disease symptoms such as stunting - survive and serve as a source of inoculum for immature *H. obsoletus* feeding on the roots. *Ranunculus bulbosus*, on the other hand, is highly susceptible and infected plants die within a few weeks. Therefore, only healthy plants of this species are available for the planthopper larvae. Infestation levels of *H. obsoletus* on *U. dioica* were usually also low in Germany. Following the recent expansion of the vector on this host plant, the proportions of infected planthoppers are progressively increasing (Fig. 4) and reach now levels of more than 30 %.

The epidemic system of BN includes wild host and reservoir plants, the vector, and grapevine as a cultivated plant. Due to the adaptation of *H. obsoletus* populations to particular host species and the association of different strains of the pathogen to such hosts the system gets even more complicated. A study carried out in 2005 in an area where both host plants and

Figure 4: Proportions of adult *H. obsoletus* infected by Bois noir phytoplasma type I in a population on nettle in the Palatinate area.
phytoplasma-strains coexist revealed that 97 % of PCR-positive *H. obsoletus* from *C. arvensis* were infected by type II of the phytoplasma while 99 % of the infected vectors from nettle carried type I of the pathogen. This leads to separate epidemiological cycles of Bois noir that include different host plants and distinct vector populations. All recent new outbreaks of Bois noir in Germany were caused by the ‘nettle system’ of the disease that includes nettle as the source of inoculum and type I of the BN-phytoplasma. This nettle cycle is still rapidly spreading and causes epidemic outbreaks of the disease for at least four years now whereas the ‘bindweed-cycle’, being the traditional system in German viticulture, remained in an endemic period characterised by decreasing disease incidence.

**Approaches to control *Hyalesthes obsoletus***

All attempts to control the spread of BN and the increase of disease incidence are aimed at the interruption of the epidemic cycles in order to decrease infection pressure on grapevine. They are hampered by the complex transmission system and the fact that both the vectoring planthopper and the phytoplasma are not restricted to grapevine and occur outside the vineyard environment, too. Possible activities include the direct control of the vector, indirect measures by controlling its host plants, and the habitat management of non-cultivated areas in order to reduce their suitability for both the vector and the host plants of BN-phytoplasma.

**Direct control of *H. obsoletus***

Insecticide treatment of grapes is not appropriate to control *H. obsoletus* which lives and feeds only occasionally on vines and occurs also outside the vineyards (Pavan, 1989; Sforza & Boudon-Padieu, 1998). On the other hand, the experimental application of a systemic insecticide on nettles in early spring, while the nymphs of *H. obsoletus* were still feeding on the roots, succeeded in the significant reduction of numbers of emerging adults in June. It also proved that the use of emergence traps is an appropriate technique to test the efficiency of control strategies for *H. obsoletus* in the field. A significant reduction of the numbers of emerging planthoppers in summer could also be achieved by plowing in winter when cold weather had been anticipated. The hibernating nymphs of *H. obsoletus* were moved to the surface and subsequently killed by frost. Laboratory experiments revealed the antagonistic potential of the entomopathogenic fungus *Metarhizium anisopliae* against *H. obsoletus* (Langer et al., 2005), however, no field experiments have been carried out so far.

**Indirect control of *H. obsoletus***

Host plants influence density and distribution of *H. obsoletus* substantially (Fig. 5). Control of those plants should therefore provide a means to reduce the vector density. To test this hypothesis, nettles were treated with herbicides in April when the planthopper nymphs still depended on this food supply. Individual nettle stands were treated selectively inside a vineyard. The numbers of adults caught on sticky traps that were positioned over treated and untreated stands were compared. In addition, larger stands of nettle plants along the border of the same vineyard were treated and the numbers of emerging adults were recorded with emergence traps. The numbers of trapped insects were reduced by 73 and 80 %, respectively, on the treated plants compared to untreated controls. The timing of weed control measures is of vital importance. If weeds are destroyed during the flight period of *H. obsoletus*, either by chemical or mechanical means, the adult vectors are forced to fly onto grapevine which results in an increased infection pressure. Weed control should be omitted from approximately four weeks before the start of the flight until its end.
**Habitat management**

An alternative to control measures is the management of the habitat inside and outside the viticultural areas in order to reduce its suitability for the vector-host-plant system that is necessary to maintain the BN-phytoplasma in the field. A well-managed green cover inside vineyards reduces its attractiveness for *H. obsoletus* that prefers open soils and at the same time reduces the density of the less compatible host plants of the vector. Uncultivated areas such as fallow vineyards in xerothermic conditions are characterized by more or less open soil covered only by sparse spontaneous vegetation and provide optimal conditions for *H. obsoletus*. We tested a mixture of seeds of various herbs that grow well in those conditions and cover the soil densely without any further tending. *C. arvensis* and *U. dioica* are efficiently suppressed by this green cover and the infection pressure from those areas is significantly decreased.

The Bois noir phytoplasma as an endemic pathogen is widespread in European ecosystems. The control measures described in this paper are helpful together with appropriate viticultural measures to reduce infection pressure and minimize economic damage. However, one should not expect to be able to eliminate the disease from areas where the environmental and viticultural conditions are favourable.

**References**


Credi, R., Terlizzi, F., Milanesi, L., Bondavalli, R., Cavallini, G., Monterini, A. & Dradi, D.


Management and biological control of wireworm populations in Europe: current possibilities and future perspectives

Lorenzo Furlan
Department of Agronomy, Entomology, University of Padova, Agripolis, via Romea 16, Legnaro PD; Italy E-mail: lorenzo.furlan@inwind.it

Abstract: To achieve a rational protection of sensitive crops from wireworm attacks in the framework of biological control, an overall strategy, taking into consideration all the information available, should be implemented. The strategy should be divided into 2 phases:

1) precise *Agriotes* population monitoring and damage prediction;
2) *Agriotes* population management which should be divided into: A) agronomic strategies: altering rotation, tillage timing, irrigation timing according to the life cycle of each *Agriotes* species, etc.; B) application of biological tools.

The most effective biological control strategy is planting the sensitive crops where no high wireworm populations are present. Currently, it is possible to predict wireworm population levels with reliable results and low costs using the pheromone traps suitable for monitoring all the most important *Agriotes* species in Europe and then select the fields without any risk of wireworm damage to plant sensitive crops. When planting of a sensitive crop has to be done in fields with high wireworm populations regardless the risk for plant stand, the available tools on the market considered active against wireworms are limited. An assay carried out in 2006 to evaluate products available in the Italian market showed that currently only the biocidal defatted seed meals have a potential to reduce wireworm populations and to protect the crop. Biofumigation caused by the biocidal defatted seed meals proved to be as effective as the chemical treatment.

Key words: wireworms, *Agriotes*, biological control, pheromone traps, biocidal seed meals

Introduction

To achieve a rational protection of sensitive crops from wireworm attacks an overall strategy, taking into consideration all the information (species identification and distribution, biology-ecology, evaluation of population levels, economic thresholds, control tools) available for different *Agriotes* species (genus including the most important wireworms) should be defined for the different conditions and implemented. The strategy should be divided into 2 phases:

1) precise *Agriotes* population monitoring and damage prediction;
2) *Agriotes* population management which should be divided into: A) agronomic strategies: altering rotation, tillage timing, irrigation timing according to the life cycle of each *Agriotes* species, etc.; B) application of biological tools.

Precise *Agriotes* population monitoring and damage prediction

The most effective strategy is planting the sensitive crops where no economic wireworm populations are present; currently it is possible to predict wireworm population levels with reliable results and low costs using pheromone traps (Furlan et al., 2001a, Karabtsas et al., 2001; Tóth et al., 2001) suitable for monitoring all the most important *Agriotes* species in Europe (*Agriotes sordidus* Illiger, *A. brevis* Candèze, *A. lineatus* L., *A. sputator* L., *A. obscurus* L., *A. rufipalpis* Brullè, *A. proximus* Schwarz, *A. litigiosus* Rossi, *A. ustulatus*...
Schäller). In last years research has provided useful information about the biological significance of pheromone trap catches and the determination of the actual range of attractiveness. Larval population levels appear correlated with click beetle capture levels estimated by using YATLORf traps in previous years (Furlan et al. 1997; 2001b, c). It is already possible to define reliable thresholds for several species and agronomic conditions. First threshold values expressed as beetles/trap/season are available for *A. ustulatus*, *A. sordidus*, *A. brevis*, *A. litigiosus* and maize crop. No matter the previous crop seasonal beetle populations of these species below 200 beetles/trap/season resulted in negligible wireworm populations and in the absence of a considerable damage to maize crop in more than 99% of the studied sites. No economic reduction of maize stand was observed anyway. To make the prediction of population levels and the actual risk of damage more reliable, agronomic and climatic characteristics of a field along with all information on biology and ecology of each species (Furlan, 1996, 1998; 2004; Rusek, 1972; Kosmacevskij, 1955) should be taken into consideration. Mathematical models (for example geostatistical analyses) may provide a good interpretation of the spatial dynamics of *Agriotes* species in relation to agronomic and geographic variables and then reduce monitoring costs (Burgio et al., 2005). Virtually non-sensitive or low sensitive crops can be planted in infested fields, while the remaining cultivated soils may be planted with any other crop. Rotation and correct allocation of the crops within a farm might be sufficient to avoid economic damage to the crops without using any specific control tool. In order to identify more precisely the areas with wireworm populations above the threshold in fields where the sex pheromone traps have detected high beetle population densities, the bait traps for larvae (Chabert & Blot, 1992, Parker, 1994, 1996) can be used. The first indicative thresholds for the bait traps were expressed as number of larvae caught by bait trap (Chabert & Blot, 1992). *Agriotes* species showed a different response to bait trap so it is necessary to assess thresholds for each of the wireworm species (Furlan, unpublished data). In Italy for maize, data collected over 15 years allowed for the defining of significant correlations between the number of larvae per sq m or the average number of larvae per bait trap and the number of damaged maize plants by *A. brevis*, *A. sordidus*, *A. ustulatus* (the threshold for this species is 3-4 times higher than *A. brevis* - Furlan, unpublished data).

**Future perspectives:** the definition of practical economic thresholds referred to pheromone traps should be extended to all the most common cases (association of: target crop, *Agriotes* species, agronomic (mainly rotation) and climatic conditions) in Europe. In order to shorten the time needed to define all the different specific thresholds it will be useful to complete the studies on the susceptibility of the crops (Furlan & Toffanin, 1996) to wireworm attacks and to link the susceptibility of the more investigated crops to the other ones. This should be associated to the information coming from an accurate study of the life table: abiotic factors seem to be the main cause of wireworm mortality but also the influence of predators (Carabidae, Stafilinidae,….) and parasites of the wireworms should be carefully investigated in order to evaluate the actual damage potential of wireworm populations so that the general thresholds might be adjusted according to the predator or parasite population levels.

**Agriotes population management**

Where population assessments suggest densities above threshold effective management strategies should be implemented. These include: A) **agronomic strategies**: altering rotation, tillage timing, irrigation timing according to the life cycle of each *Agriotes* species, etc. B) **application of biological tools**.
Agronomic strategies
Crop rotation and availability of food resources through the season, climatic-agronomic conditions (mainly organic matter content) and soil characteristics are the main factors influencing the composition of species communities and larval population density. For the species studied in Italy, the most important factor appears to be crop rotation (Furlan & Talon, 1997; Furlan et al., 2000); this is the situation in other regions as well (e.g. Szaruka, 1977). The presence of meadows and double cropping within the rotation cycle results in a population increase of species overwintering as adults (Furlan, 2005). Therefore, any modification of these factors may result in a variation of Agriotes population dynamics.

Altering rotation: the temporary interruption of the most suitable rotation for wireworm development is a powerful tool for biological control.

Tillage timing: choosing a rotation and/or crops in the rotation that allow to do soil tillage in the most critical phase of the life cycle of the click beetles (e.g. prevalence of eggs and first instar larvae in the soil) may dramatically reduce wireworm populations; this should be modulated according to the differences in the life cycle of the main Agriotes species.

Irrigation timing: the drying up of the top soil layer just after egg laying is a primary mortality factor; avoiding irrigation in this phase can heavily reduce Agriotes populations.

Planting time: the capacity of damaging sensitive plants varies with the seasonal period; for example in late spring, very high A. ustulatus populations cannot damage maize stands because most of the larvae are in a non-feeding phase (Furlan, 1998).

Intercropping with wheat or other plants may also be included in the control strategy (Furlan & Toffanin, 1994; Vernon et al., 2000).

Application of biological tools
Currently, where economic wireworm populations have been found and there is no possibility of moving the sensitive crop to non-infested fields, the only effective biological protection options seem to be the biocidal plants and the biocidal defatted seed meals. In spring 2006 the products regarded as useful tools for protecting crops from wireworm attacks in organic farming and available in the Italian market were tested in semi-natural conditions (commercial entomopathogenic nematodes were available as well but previous trials had excluded any significant control effect). The trials were performed in plastic pots with upper diameter 14 cm and volume 1,1 l. The holes in the pot bottom were closed with cotton tissue to prevent larvae from escaping and to allow, at the same time, water in excess dropping out. Each pot was filled with sandy soil kept at maximum water content. Meals were mixed up with the soil. In each pot, just after soil treatment, 8 larvae were introduced. The trial was arranged in a randomized block design with four replications placed in a shadowy place, evaluating the following treatments:

Untreated: 2 non coated maize seeds per pot – no treatment in the soil

Untreated + L: as above + wireworms

Biocidal meal: 2 non coated maize seeds per pot + 1,0 g/l of carinata defatted seed meal (Biofence) mixed up with soil equivalent to 20 q/ha incorporated in the uppest 20 cm

Biocidal meal + L: as above + wireworms

Ricinus seed meal: 2 non coated maize seeds per pot + Manna ricino coarse, ricinus seed meal, not declared Ricinine content, dosage 2,5 g/l equivalent to 50 q/ha incorporated in the uppest 20 cm of the soil

Ricinus seed meal + L: as above + wireworms

Beauv: 2 non coated maize seeds per pot + commercial product Naturalis (declared content of Beauveria bassiana ATCC 74040 7,16 %) at the dosage of 1 cc/sq m

Beauv+L: as above + wireworms

Neem cakes: 2 non dressed maize seeds per pot + GreenNeem cake - declared Azadirachtin
content 800-900 ppm - dosage 0,5 g/l equivalent to 10 q/ha incorporated in the top 20 cm
Neem cakes + L: as above + wireworms
Imidacloprid: 2 maize seeds coated with imidacloprid at the rate of 1,25 mg a.i./seed
Imidacloprid + L: as above + wireworms.

Each pot was irrigated after treatment and inspected 14 days after larvae introduction. The results were evaluated counting healthy or damaged emerged maize plants. Later, seeds and plants were taken off and evaluated to check the presence of scars and holes caused by feeding activity of the larvae. Finally the pot soil was put on a plastic sheet in order to find and count the larvae. Recovered larvae were divided into 3 groups: i) alive and mobile, ii) slowly mobile and iii) dead. Experimental data were transformed to square root (x+0.5) to ensure homogeneity of variances and then evaluated utilizing analyses of variance (ANOVA) and Tukey’s mean separation test.

Results of the experiment are summarized in Table 1. Biocidal defatted seed meals (from *Brassica carinata* sel. ISCI 7) proved to be as effective as the chemical treatment while all the other biological treatments did not cause any larval mortality and plant protection. The insecticidal effect due to the volatiles produced by the glucosinolate enzymatic hydrolysis of the defatted seed meals confirmed the results of previous research which demonstrated the potential of biocidal plants (*Brassica juncea* sel. ISCI 99) as well (Furlan et al., 2004). The biocidal meals in last two years proved to be effective in open field conditions as well providing long term *Agriotes* control by reducing the different larval instar populations and even protecting the sensitive crop just before planting like the most effective soil insecticides. The other biological treatments confirmed the ineffectiveness showed in previous years (Furlan, unpublished data); as to the insect pathogenic fungus used the mortality of the treated larvae was low also some months later.

*Future perspectives:* other potential biological tools such as the entomopathogenic fungus *M. anisopliae* should be evaluated under semi-natural and field conditions.

Table 1: Effect of available biological products on wireworm populations and crop protection.

<table>
<thead>
<tr>
<th></th>
<th>emerged plants</th>
<th>alive larvae</th>
<th>scars on seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>2,00 d</td>
<td>0,00 a</td>
<td>0,00 a</td>
</tr>
<tr>
<td>Untreated+L</td>
<td>0,75 abcd</td>
<td>5,75 b</td>
<td>3,50 c</td>
</tr>
<tr>
<td>Biocidal meal</td>
<td>1,50 bcd</td>
<td>0,00 a</td>
<td>0,00 a</td>
</tr>
<tr>
<td>Biocidal meal + L</td>
<td>1,75 cd</td>
<td>0,75 a</td>
<td>0,13 a</td>
</tr>
<tr>
<td>Ricinus seed meal</td>
<td>0,75 abcd</td>
<td>0,00 a</td>
<td>0,00 a</td>
</tr>
<tr>
<td>Ricinus seed meal+L</td>
<td>0,00 a</td>
<td>6,33 b</td>
<td>3,67 bc</td>
</tr>
<tr>
<td>Beauv</td>
<td>1,75 abcd</td>
<td>0,00 a</td>
<td>0,00 a</td>
</tr>
<tr>
<td>Beauv+L</td>
<td>0,75 abcd</td>
<td>6,00 b</td>
<td>4,75 c</td>
</tr>
<tr>
<td>Neem cakes</td>
<td>1,50 bcd</td>
<td>0,00 a</td>
<td>0,00 a</td>
</tr>
<tr>
<td>Neem cakes + L</td>
<td>0,25 ab</td>
<td>5,25 b</td>
<td>4,13 c</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>0,75 abcd</td>
<td>0,00 a</td>
<td>0,00 a</td>
</tr>
<tr>
<td>Imidacloprid + L</td>
<td>1,50 bcd</td>
<td>3,75 b</td>
<td>0,38 ab</td>
</tr>
</tbody>
</table>
Conclusions

Based on the information supplied above, a rational procedure to avoid wireworm damages to sensitive crops without any chemical applications might be the following:

A) locate the areas with high and low risk for wireworm attacks by considering agronomic factors and sex pheromone trap captures;
   A1) Low risk areas: plant sensitive crops;
   A2) High risk areas: continue Agriotes population level evaluation;

B) among areas with high risk for wireworm attacks, locate areas with actual Agriotes populations above threshold by using the bait traps for larvae:
   B1): zones with low wireworm population (not economic) found: planting sensitive crops in these areas is possible without any treatments.
   B2): zones where high (economic) wireworm populations have been found:
      B2a): no sensitive crops in the same season or application of biocidal defatted seed meals or incorporation of biocidal plants if sensitive crops are going to be planted;
      B2b) if sensitive crops are going to be planted late in the season or in next year two options may be implemented:
         - Interfering with Agriotes life cycle (e.g. by tilling the soil in the most suitable period to cause high mortality - maximum presence of eggs and newly hatched larvae)
         - application of biological treatments like biocidal plants or seed meals during population development (rotation may be planned taking these aspects into consideration too) or just before sensitive crop sowing.

Acknowledgements

I would like to thank Dr Luca Lazzeri, Dr Giampiero Patalano, Dr Christian Bonetto and p.a Andrea Finotto for the contribution to pot trials.

References


Illiger in Modelli Agricoli e Impatto Ambientale, valutazioni aziendali e territoriali, Raisa, UNIPRESS, Padova, 11-16.


Furlan, L. 2005: An IPM approach targeted against wireworms: what has been done and what still has to be done. IOBC/wprs Bull. 28(2): 91-100.


Wireworms
Occurrence of click beetle pest spp. (Coleoptera, Elateridae) in Europe as detected by pheromone traps: survey results of 1998-2006

Lorenzo Furlan 1, Miklos Toth 2, Cooperators 3
1Department of Agronomy, Entomology, University of Padova, Agripolis, via Romea 16, Legnaro PD; Italy; 2 Plant Protection Institute, HAS, Budapest, Herman O. u. 15, H-1022 Hungary; 3 see Acknowledgements

Abstract: The implementation of IPM strategies against wireworms has been very difficult because of the shortage of reliable information on the key aspects of the species until few years ago. One of these key aspects is represented by the species distribution over the different European regions. Knowing the species present allows us to establish promptly oriented monitoring programs saving time and materials and a general prediction of damage risk for the sensitive crops based on the knowledge of biology and behaviour of the different Agriotes species. A reliable description of the distribution of the main Agriotes species is currently possible because pheromone traps suitable for monitoring all of the most important Agriotes species in Europe are available. They proved to be effective to detect the presence of species also at very low population levels. The maps of the distribution of the main Agriotes species in Europe are presented and commented.

Key words: sex pheromone traps, wireworms, Elateridae, Agriotes brevis, A. litigiosus, A. lineatus, A. obscurus, A. rufipalpis, A. sordidus, A. sputator, A. ustulatus, A. proximus, Coleoptera, IPM strategies

Introduction

The implementation of IPM strategies against wireworms has been very difficult because of the shortage of reliable information on the key aspects of the species until few years ago. One of these key aspects is represented by the species distribution over the different European regions.

In some regions, general historical information about harmful wireworm species is available (Dolin, 1978; Furlan et al., 2000; Rusek, 1972); yet in most cases, no precise data about the actual distribution of species in different rural areas are available. Since behavioural differences between species can occur and damage to crops can happen at different times during the growing season, precise information on the species present within each area would be useful. Knowing the species present allows to establish promptly oriented monitoring programs saving time and materials and a general prediction of damage risk for the sensitive crops based on the knowledge of biology and behaviour of the different Agriotes species.

Materials and methods

Adult monitoring

Over a 9 year period (1998 - 2006) YATLORf sex pheromone traps (Furlan et al., 2001a) have been deployed in several European regions. They have been baited with the lures attracting the most important Agriotes species: A. sordidus Illiger and A. rufipalpis Brullè, A. proximus Schwarz and A. lineatus L., A. ustulatus Schäller, A. litigiosus Rossi, A. brevis Candeze, A. sputator L., A. obscurus L. (Furlan et al., 2001a; Tóth et al., 2003). The following methods were used:
Cap Position: low for *A. brevis* and *A. obscurus*, medium for *A. sordidus/rufipalpis*, *A. sputator*, *A. lineatus/proximus* and high for *A. ustulatus*, *A. litigiosus* in the same type of trap. 

Replacement of the Caps: every 30-40 days except for *A. brevis* (no change).

Inspections: generally twice per week, sometimes once per week.

Period of Monitoring: from early March (*A. brevis, A. rufipalpis*) - April until August 30.

Trap Position: in the middle of monitored fields as shown in Figure 1.

![Figure 1: Trap layout for monitoring.](image)

- = bait trap and soil sampling (1 – 2 m from the bait trap)
- A. sordidus/rufipalpis, A. lineatus, A. obscurus sex pheromone trap
- = other sex pheromone traps (*A. litigiosus, A. ustulatus, A. sputator, A. brevis*)

**Larval population estimation**

This was done in parts of the monitored sites in early spring or autumn, when soil temperatures were above 10°C. A 5 x 10 grid (20 m x 30 m, Fig. 1) of soil samples was taken covering the area where each pheromone trap had been set up. Each soil sample was 12 cm in diameter and 30 to 60 cm deep according to the season. Bait traps were placed 1-2 m away from each point where the soil cores were taken or would be taken, provided the soil was bare. Each trap was made and used according to the description given by Chabert and Blot (1992).

**Results**

The sex pheromone traps proved to be effective in detecting the presence of species, even at very low population levels, and made it possible to draw comprehensive maps of species distribution (Figures 2 to 8). Data are in agreement with those already available (Furlan et al., 1997; Furlan et al., 2001a, 2001b; Karabatsas et al., 2001; Tóth et al., 2001; Tóth et al., 2005). Some species are restricted to defined areas (e.g. *A. ustulatus* in central-eastern Europe, *A. litigiosus* in the south, *A. sordidus* in the south being replaced by *A. rufipalpis* in the most south-eastern part) while others (e.g. *A. lineatus*) are widespread almost everywhere. The same sex pheromone can capture different targeted species (e.g. geranyl hexanoate actively attracts both *A. sordidus* Illiger and *A. rufipalpis* Brullé) or non targeted species (e.g. *A. sputator* lures can capture *A. acuminatus* Stephens). Based on this information it is possible, for practical purposes, to place out few specific traps at each studied region. Life cycles differ dramatically between species (Furlan, 2005). Detailed information on the biology of some species is available (Furlan, 1996, 1998, 2004, 2005; Furlan et al., 2004) and allows to predict the risk of damage and to implement the most effective IPM strategies.
Knowing which species are really present in an area allows the implementation of IPM strategies with low, sometimes without any, monitoring efforts. Despite the fact that correlation between beetle captures and subsequent larval populations is influenced by many biotic and abiotic factors and many variables are involved, it was possible to get first reliable data, e.g. larval captures occurred only where high beetle captures - more than 2-300/season - were detected corroborating preliminary data obtained (Furlan et al. 1997, 2001b).

Figure 2. Catches of click beetle spp. in traps with the synthetic pheromone bait of *A. brevis* in different European countries between 1998 - 2006.

Figure 3. Catches of click beetle spp. in traps with the synthetic pheromone bait of *A. lineatus* in different European countries between 1998 - 2006.
Figure 4. Catches of click beetle spp. in traps with the synthetic pheromone bait of *A. litigiosus* in different European countries between 1998 - 2006.

Figure 5. Catches of click beetle spp. in traps with the synthetic pheromone bait of *A. obscurus* in different European countries between 1998 - 2006.

Figure 6. Catches of click beetle spp. in traps with the synthetic pheromone bait of *A. sordidus / A. rufipalpis* in different European countries between 1998 - 2006.
Figure 7. Catches of click beetle spp. in traps with the synthetic pheromone bait of *A. sputator* in different European countries between 1998 - 2006.

Figure 8. Catches of click beetle spp. in traps with the synthetic pheromone bait of *A. ustulatus* in different European countries between 1998 - 2006.

**Conclusions**

The sex pheromone traps proved to be effective in detecting the presence of *Agriotes* species, and made it possible to draw comprehensive maps of species distribution that allow us:

- to start, for practical purposes, with a precise monitoring exclusively of the *Agriotes* species potentially present in each European country, saving money and time;
- to identify easily the species damaging the crops in individual fields;
- to assess the potential risk of damage for different sensitive crops based on the information on the biology available for the species present in the different regions.
Acknowledgements


References

Furlan, L. 2005: An IPM approach targeted against wireworms: what has been done and what still has to be done. IOBC/wprs Bull. 28(2): 91-100.
Spatial distribution of click-beetles (Agriotes spp.) at field and landscape scales

Rod P. Blackshaw¹, Robert S. Vernon², Helen Hicks¹
¹School of Biological Sciences, University of Plymouth, Drake Circus, Plymouth PL4 8AA, UK;
²Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Agassiz Research Station, P.O. Box 1000 – 6947, #7 Highway, Agassiz, British Columbia V0M 1AO, Canada. E-mail: rblackshaw@plymouth.ac.uk

Abstract: Pheromone traps were used to study the spatial distribution of the click-beetles Agriotes lineatus and A. obscurus in the closed agricultural landscape of Westham Island, British Columbia, Canada over a three year period. Spatially referenced counts were analysed using Spatial Analysis by Distance Indicators (SADIE). This showed that there were statistically significant patches of higher, and gaps of lower, counts. SADIE association analyses were applied and it was concluded that there was considerable temporal stability for both species at this scale, both between and within years, and that both species had similar spatial patterns. This study was extended to the smaller field scale at two sites in British Columbia, Canada. There was considerably less evidence for spatial stability and different patterns emerged for each species. The observed distribution patterns were dependent on the distance between pheromone traps and it was postulated that A. lineatus either moved faster, or was more responsive to pheromones, than A. obscurus. This hypothesis was tested in a mark release recapture field trial in England and was accepted. It is concluded that simultaneous counts of different species of click-beetles in pheromone traps cannot be uniformly interpreted.

Key-words: Agriotes, pheromone trapping, SADIE, spatial dynamics, mark release recapture

Introduction

An appreciation of the spatiotemporal ecology of invertebrates in agricultural systems is an increasingly important component of the development of integrated pest management strategies. Historically the focus of such studies has been the field population even though this is a human construct that may not be the delineating characteristic of a population (Holland et al. 2005). Such factors as non-farmed habitats within the agricultural landscape may be important for sustaining many pest species, especially soil-dwelling insects such as wireworms that are susceptible to cultivation mortality.

There are now over 7000 sex pheromones and semiochemicals identified and synthesised for insect control (El-Sayed 2005), and the research area, as expressed by published papers, is accelerating (Boo & Park 2005). Improvements in both technology and methodology have resulted in a situation where the generation of pheromone-based monitoring capacity is driven by the identification of distinct sex pheromones for pest species, rather than an understanding of the dynamics of the captured adult males in relation to those of the larvae. Pheromone monitoring systems for Agriotes spp. have been developed (Toth et al. 2003) and are currently deployed to predict likely damage by larval feeding (Anon 2006) despite there being little verifiable data available to define any relationships. (Also see Furlan, this issue)
In this paper we report two studies of the spatiotemporal distribution of *A. lineatus* and *A. obscurus* in an agricultural landscape and within two fields, and a mark release recapture study to test a resultant hypothesis.

**Material and Methods**

The same pheromone traps were used in both the landscape and field studies. The traps (c. 15 cm x 15 cm) were Vernon Beetle Traps (Phero Tech Inc. Delta, BC) constructed of durable PVC, and designed to capture and confine adult beetles that are attracted to the internal pheromone lure and fall in after ascending shallow ramps (Vernon 2004). The traps were placed at ground level with entry ramps either flush with the ground, or slightly covered by soil to provide unimpeded beetle entry. Recovery of the beetles was by removal of one of the two parallel ramps so that specimens could be shaken out on to a tray. No preservative was used in the traps.

The pheromones were dispensed from single bubble caps (Phero Tech Inc, Delta, BC). For *A. lineatus* the dispensers contained 160 mg of geranyl octanoate and geranyl butanoate in a 9:1 ratio, whilst for *A. obscurus* the mixture was 160 mg of geranyl octanoate and geranyl hexanoate in a 1:1 ratio.

The landscape studies were carried out on Westham Island, BC, Canada which lies in the mouth of the Fraser River. This island covers some 950 ha of mixed farming and conservation land. Paired pheromone traps separated by approximately 20 m were set out at a number of locations in non-farmed habitats across the island. Traps were operated from April to July in 2002, 2003 and 2004 and emptied every three weeks.

For the within field studies, two fields in the Fraser Valley were used. At the time of sampling in 2002 both fields were in commercial strawberry production. The crop at Site 1 had been established in 1999; prior to this the field had been in grass for more than 10 years. The field had rough grass along the eastern and southern boundary, and farmed grass along the western and northern boundaries separated by a hedgerow. The crop at Site 2 was planted in 2000 and was immediately preceded by a long-term field of hops (>20 years). It was located within a larger cultivated area.

At Site 1, 101 bait traps (modified from a trap reported in Kirfman et al. 1986) were placed every 15.9 m along parallel transects 24.5 m apart on 21 May 2002. Traps were recovered and replaced on 4 June 2002, and the second set recovered on 18 June 2002. A similar study was conducted at Site 2, with 96 bait traps placed every 34.7 m along transects at 48 m spacing on 24 May 2002. Cumulative counts of *Agriotes* spp. were used in subsequent analyses.

Pheromone traps for the two target species were placed in each field alternately (except for one row at Site 1 where two pairs of traps were inadvertently transposed) at each of the bait trap locations resulting in 51 *A. lineatus* and 50 *A. obscurus* sampling points in Site 1, and 48 of each in Site 2. Site 1 traps were deployed on 20 March 2002, and Site 2 traps on 5 April 2002. Traps were emptied, approximately every two weeks until 9 July 2002, and click beetles identified to species.

For all these adult and larval data, spatial analyses were carried out using Spatial Analysis by Distance Indices (SADIE) described by Perry (1998) and Perry et al. (1999). This set of methods provides a dimensionless analytical tool that enables statistical comparison of the spatial information arising from different point counts.

In early summer 2006, adult males of the two click-beetle species were captured in pheromone traps, and marked. They were released in a grass field in south Devon, UK at distances (4, 8, 16 and 32 m) and directions (North, South, East and West) from a new
pheromone trap. Numbers of individuals released were increased with distance (x1, x2, x4, x8) to improve chances of recapture. Data were interpreted as proportionate cumulative recovery over time.

Results and Discussion

*Agriotes lineatus* was clustered into patches and gaps at the landscape scale in all three years, and *A. obscurus* showed similar spatial structure in 2002 and 2003. For both species, spatiotemporal stability at the landscape scale was evident. There was also a positive association between the two species in two out of three years, suggesting that they were responding to the landscape environment in a similar manner. These results affirm that non-farmed habitats may be important to maintaining click-beetle spatial distributions over time in agricultural landscapes. For a full report see Blackshaw and Vernon (2006).

Contour plots of counts at the two field sites were made using the kriging function in SURFER® (Figs 1 and 2). For *A. lineatus* there was no statistically significant spatial structure apparent at either site after the first sampling date in early May, whereas *A. obscurus* showed significant spatial structure over the first three sampling occasions at Site 1 and on all but one occasion at Site 2. Similar differences between the two species were also seen when SADIE association tests were conducted between cluster indices for different sampling occasions. We also note, especially in Site 1 (Fig 1), that there is visual evidence of a field edge effect, reinforcing the hypothesis that non-farmed habitats are important to the maintenance of *Agriotes* populations.

These results suggest that population spatial structure detectable by pheromone trapping is a function of time and trap spacing. Both species exhibit significant structure early in the sampling period but this is maintained longer for *A. obscurus* than for *A. lineatus*.

Spatial association tests between adult and larval SADIE cluster indices showed differences between the two species. At Site 1, *A. lineatus* males were significantly dissociated from larval spatial distributions on the first two sampling occasions with a tendency to dissociation on other occasions. Similarly *A. obscurus* showed this tendency to dissociation although less pronounced with the only statistically significant effect on the first sampling date. The situation for Site 2 differed. *A. lineatus* showed the same tendency towards dissociation as at Site 1 except for the last sampling date when there was a statistically significant association with wireworm numbers. In contrast, *A. obscurus* distributions were significantly associated with wireworm distributions on all sampling dates at Site 2.

A limitation to interpretation of these results is the lack of knowledge about which *Agriotes* species were present in the wireworm population. However, it is clear that any relationship between pheromone and bait trap counts will differ for the two species.

The observed differences in spatial structure for *A. lineatus* and *A. obscurus* can be related to the spacing of traps, and become more pronounced the closer the traps. There was essentially no difference detected at the landscape scale, little initial difference at Site 1, and pronounced difference at Site 2 where traps were four times as dense as at Site 1. One interpretation of this is that *A. lineatus* either moves faster or responds more readily to pheromones than does *A. obscurus*. The mark release recapture study showed that a greater proportion of *A. lineatus* were recovered and in a shorter time (Fig 3). Thus we conclude that the two species do indeed show different behavioural responses.
Fig 1. Contour maps of wireworm counts and pheromone trap count data for different sampling dates at Site 1. The figures are orientated so that east is at the top.
Fig 2. Contour maps of wireworm counts and pheromone trap count data for different sampling dates at Site 2. The figures are orientated so that south is at the top.
Fig 3 Proportion of marked click-beetles recovered over time in pheromone traps following release at 4 (●), 8 (■), 16 (▲) and 32 (×) m distance. The plotted values are the means of four release points.

The implication of these results for pheromone trapping as a monitoring tool is that the count of click-beetles over time means something different for each species. In this context, the UK advisory practice of summing counts for *A. lineatus*, *A. obscurus* and *A. sputator* in order to predict potato damage by wireworms has no scientific validity.

Acknowledgements

The authors thank Marcus Clodius for helping to assemble the data. The work in Canada was part of a larger programme funded by Agriculture and Agri-Food Canada, Fraser Valley Strawberry Growers Association, Potato Industry Development Committee, Canadian Wildlife Service, Environment Canada, Ducks Unlimited Canada, Waterfowl Society, E.S. Cropconsult Ltd., Phero Tech Inc., Canadian Food Inspection Agency, BCARC, University College of the Fraser valley, Lower Mainland Horticultural Improvement Association, Bayer AgroScience, Syngenta, and Zeneca Agro. The Perry Foundation, South Devon Organic Producers and DEFRA supported the UK work through the Sustainable Arable LINK programme.

References

Anon 2006: http://www.syngenta-crop.co.uk/What+is+Happening/Press+Releases/10-02-2005.htm Accessed 24/04/06


European wireworms (*Agriotes* spp.) in North America: toxicity and repellency of novel insecticides in the laboratory and field

Robert S. Vernon, Wim Van Herk, Chandra Moffat, Chantelle Harding
*Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, P.O. Box 1000, Agassiz, British Columbia, CANADA V0M 1A0.*

**Abstract:** Three species of wireworms, notably *Agriotes obscurus*, *A. lineatus*, and *A. sputator*, were introduced to North America from Europe about a century ago. All three species are present in the Atlantic provinces of Eastern Canada, and *A. obscurus* and *A. lineatus* are present in the westernmost province of British Columbia, and in the states of Washington and Oregon in the USA. These species and several other endemic species have become major pests of a variety of crops across Canada and the USA, with their major impact being on potatoes, corn and cereals. Of major concern in Canada is that all of the most commonly used insecticides for wireworm control are no longer available, and attempts to register new and effective wireworm insecticides are proving to be difficult from both regulatory and biological points of view. Due to the complex subterranean activities of wireworms, and the time and expense required for wireworm sampling, contemporary efficacy studies often rely solely on crop stand, yield and/or cosmetic injury criteria when evaluating new insecticides. By extrapolation, circumstantial evidence of wireworm damage is often equated with reductions in wireworm populations. Although this assumption was likely correct with the older organochlorine and organophosphate chemistries (we have evidence for this), we have found that this does not apply to many of the newer chemistries being studied and registered for wireworm control. Studies in the laboratory and field, for example, have shown that although exposure to various neonicotinoids (i.e. thiamethoxam, clothianidin and imidacloprid) will suppress damage (i.e. corn, wheat) by several economic species of wireworms, this is due primarily to wireworms entering a rapid and long-term state of intoxication rather than mortality. Exposed populations recover later on in the growing season. In contrast, fipronil (a phenyl pyrazol) applied at higher dosages to wheat and potato crops resulted in excellent crop protection and virtual extermination of *A. obscurus* populations by the following growing season. We also found that applications of fipronil at low dosages to *A. obscurus* in the laboratory did not affect wireworm health immediately, but significant latent morbidity and mortality (up to 90%) began occurring after about 40 days. In specially-developed soil bioassays, we have also found that tefluthrin (a synthetic pyrethroid), although registered in Canada for wireworm control in corn, is actually repellent to *A. obscurus* and *Limonius canus*. Damage control with tefluthrin in corn, therefore, is likely due to wireworms being repelled rather than killed during the critical establishment stage. This paper discusses the implications of these data as they relate to the current and future screening of novel lower risk insecticides.

**Key words:** Wireworms, *Agriotes obscurus*, neonicotinoids, phenyl pyrazols, efficacy, toxicity, repellency

**Introduction**

Wireworms, the larval stage of click beetles (*Coleoptera: Elateridae*), have become one of the most serious polyphagous insect threats to many agricultural crops worldwide (Parker and Howard 2001). What makes them particularly challenging in Canada, is that several introduced and endemic species, all with unique life histories and geographic niches are involved (Glen *et al.* 1943; Becker 1956). In British Columbia, key species include the dusky
wireworm, *Agriotes obscurus* L., and the lined click beetle, *A. lineatus* L., both introduced from Europe more than a century ago (Wilkinson 1963; Vernon *et al.* 2001). In Atlantic Canada, these and an additional European wireworm, *A. sputator* L. have become the dominant economic species (Eidt 1953). In the Prairie provinces, key species include the Pacific coast wireworm, *Limonius canus* (LeConte), the sugarbeet wireworm, *L. californicus* (Mann), the prairie grain wireworm, *Ctenicera destructor* (Brown), the wheat wireworm, *A. mancus* and others. In Ontario and Quebec, *A. mancus*, the eastern field wireworm *L. agonis* (Say) and a complex of *Melanotus* spp, (e.g. *Melanotus communis*) are economically important (Glen *et al.* 1943).

Wireworms, due to their subterranean feeding habits, can cause catastrophic crop losses due to stand and yield reduction (i.e. cereals, corn, vegetables, small fruits, ornamentals) and/or cosmetic injuries (i.e. carrot, strawberry, potato)( Wilkinson 1963; Vernon 2005). Typically, wireworm problems are most severe in fields that have had a recent history of pasture or cereal crops, since these are the preferred hosts for wireworms, and are targeted oviposition sites for adult click beetles (Parker and Howard 2001; Wilkinson 1963). When these crops are removed, wireworms remaining in the field will feed on many higher value crops planted in rotation, and due to their long life cycles (2-6 years depending on the species), damage by wireworms can continue for several years.

Since WWII, wireworms have been controlled in Canada with a robust arsenal of effective chlorinated hydrocarbon (OC), organophosphate (OP) and carbamate insecticides applied to the soil and/or to seed (i.e. cereals, corn) (Wilkinson *et al.* 1964, Toba *et al.* 1985). The early use of the more persistent OCs (i.e. aldrin and heptachlor), for example, would control wireworm populations in fields for 9 years with a single application to soil (Wilkinson *et al.* 1964), and grain seed treated with the less persistent OC lindane has been used to reduce damage and wireworm populations on the prairies. In potatoes, the use of several higher risk, but lower residual OP and carbamate insecticides provided economic control following the de-registration of the more residual OCs in the 1970s. During the past decade, however, most of the effective wireworm insecticides have been de-registered on all crops in Canada, with only phorate (e.g. Thimet 15G) remaining for wireworm control on potatoes. As a result, damage has been increasing dramatically in many susceptible crops, such as in wheat and corn, and control of wireworms in potatoes is becoming increasingly unreliable with phorate. Although some attrition of wireworm insecticides has also occurred in the USA, a number of effective insecticides still remain for wireworm control on many crops, and additional new and effective chemistries have been registered (e.g. the phenyl pyrazol fipronil on corn)(Kuhar *et al.* 2003).

Due to the growing severity of wireworm problems in many of Canada’s key crops, and to the severe lack of effective insecticides relative to the USA, the development and registration of effective wireworm management products and strategies has become a research priority with Agriculture and Agri-Food Canada (AAFC). This paper describes the activities being conducted by AAFC towards the understanding and development of lower risk insecticides for wireworm control in key crops in Canada.

**Insecticide Efficacy/Toxicology**

Since 2000, considerable progress has been made by AAFC researchers in the development of laboratory and field methodologies that have been used to screen the effectiveness of lower risk insecticides for wireworm control. In laboratory studies conducted between 2004 and 2006, the long term effects of several insecticides representing 6 insecticidal classes were tested on five economic Canadian species (*A. obscurus, A. sputator, C. destructor, C.*
pruinina and L. canus). These lab studies, combined with complimentary field studies, have provided invaluable new information relating to the relative toxicities and sub-lethal effects of the leading candidate insecticides on these economically important wireworms.

**Neonicotinoids**

Of particular significance in our studies was the discovery that wireworms exposed dermally to neonicotinoids (via topical applications (LD50) and Potter spray tower applications (LC50), immediately entered into various states of long term intoxication. This was observed with a number of thianicotinoids (clothianidin and thiamethoxam) and chloronicotinoids (imidacloprid and acetamiprid), with the thianicotinoids having the lowest LC50’s and LD50’s on all species tested. Depending on the chemical and dose applied, intoxication could last several months, at the end of which either full recovery or death of the wireworms occurred. When intoxicated, wireworms could not move in a directed fashion, and were often seen writhing, or immobilized with only leg and/or mandible movement. During intoxication, feeding in the wireworm storage containers did not occur. Similar results were observed with thiamethoxam in soil-based bioassays that allowed both contact and feeding.

In the field, neonicotinoids (imidacloprid, clothianidin and thiamethoxam) applied to wheat seed generally provided excellent stand establishment and yield in *A. obscurus* infested fields between 2003 and 2006. Wheat stand in clothianidin-treated plots relative to untreated control plots, for example, is shown in Figure 1 (A) for trials conducted in 2003 and 2004. However, when wireworm trapping was conducted in the years following these trials (i.e. 2004 and 2005), the number of wireworms taken in the clothianidin treatments were not significantly different than in the untreated control treatments (Figure 1B). This trend was observed at all application rates of clothianidin tested, as well as with several rates of thiamethoxam and imidaclorpid.

![Graph 1](image1.png)

**Figure 1.** Wheat trials conducted in Agassiz, BC in fields infested with *A. obscurus*. Wheat stand in plots treated with clothianidin (Poncho 600F at 10g a.i./100kg wheat seed) relative to check plots in 2003 and 2004 (A), and wireworm populations recovered from bait traps in these same treatment plots one year later (B).

Neonicotinoid treatments (clothianidin and thiamethoxam) applied to potato seed pieces also provided excellent potato stand establishment and control of wireworm blemishes at harvest (100 days after planting) in *A. obscurus* infested fields between 2003 and 2006. The number of wireworm blemishes per tuber in clothianidin-treated plots relative to untreated
control plots is shown in Figure 2 (A) for trials conducted in 2003 and 2004. When wireworm trapping was conducted in the years following these trials (i.e. 2004 and 2005), the number of wireworms taken in the clothianidin treatments were not significantly different than in the untreated control treatment (Figure 2B). This trend was also observed with all application rates of clothianidin, as well as with seed piece treatments with thiamethoxam.

![Figure 2](image)

Figure 2. Potato trials conducted in Agassiz, BC in fields infested with *A. obscurus*. Tuber blemishes in plots treated with clothianidin (Poncho 600F at 12g a.i./100kg potato seed) relative to check plots in 2003 and 2004 (A), and wireworm populations recovered in these same treatment plots one year later (B).

Our combined laboratory and field data strongly suggest that damage suppression in wheat and potato crops treated with various neonicotinoids is due to wireworm populations becoming intoxicated upon contact with, and/or feeding on, treated seed. The data further suggest that damage protection is not due to wireworm mortality, since populations in the laboratory and field later recovered. The observation that wireworms may be providing crop protection without killing wireworms is important, especially if a key objective of using an insecticide is to reduce wireworm populations in the year(s) following.

**Phenyl pyrazols**

Another important observation during our laboratory toxicity trials in 2004 was that fipronil, a phenyl pyrazol registered for wireworm control in several countries outside of Canada, provided rapid intoxication and almost complete mortality of *A. obscurus* wireworms upon dermal contact at the highest concentrations tested. Of greater importance was that fipronil, applied dermally to *A. obscurus* at doses far below those required for any of the other insecticides tested, resulted in almost complete latent mortality of wireworms several months after exposure. Wireworms exposed to these low doses did not show any signs of intoxication for up to several months after exposure, and were observed to feed and function normally during that time.

In the same wheat field studies as reported above for clothianidin, fipronil (Regent 4SC) applied to wheat seed also provided excellent stand establishment and yield in *A. obscurus* infested fields between 2003 and 2006. Wheat stand in fipronil-treated plots relative to untreated control plots is shown in Figure 3 (A) for trials conducted in 2003 and 2004. When wireworm trapping was conducted in the years following these trials (i.e. 2004 and 2005), there were no wireworms taken in any of the fipronil treatments (Figure 3B).
In the same potato field studies as reported above for clothianidin, fipronil (Regent 4SC) sprayed in furrow at time of planting also provided excellent potato stand establishment and control of wireworm blemishes at harvest (100 days after planting) in 2003 and 2004 (Fig. 4A). When wireworm trapping was conducted in the years following these trials (i.e. 2004 and 2005), the number of wireworms taken in the fipronil treatments were significantly lower (no wireworms taken in 2005) than in the untreated control treatments (Figure 4B). The combined laboratory and field data suggest that damage suppression in wheat and potato crops treated with fipronil is likely due to wireworm populations becoming intoxicated and ultimately dying upon contact with, and/or feeding on, treated seed.

![Figure 3](image1)

Figure 3. Wheat trials conducted in Agassiz, BC in fields infested with *A. obscurus*. Wheat stand in plots treated with fipronil (Regent 4SC at 60g a.i./100kg wheat seed) relative to check plots in 2003 and 2004 (A), and wireworm populations recovered in these same treatment plots one year later (B).

![Figure 4](image2)

Figure 4. Potato trials conducted in Agassiz, BC in fields infested with *A. obscurus*. Tuber blemishes in plots treated with fipronil (Regent 4SC at 3.05 g a.i./100m row) relative to check plots in 2003 and 2004 (A), and wireworm populations recovered from bait traps in these same treatment plots one year later (B).
Insecticide Effects on Wireworm Behaviour

Agriculture and Agri-Food Canada in Saskatoon, Saskatchewan was the first laboratory to accurately describe the attractiveness of wireworms to carbon dioxide produced by germinating grain (Doane et al. 1975). Expanding on these studies, modified laboratory methodologies and models have been developed at AAFC, Agassiz to study the attractancy, repellency and toxicity of insecticides to wireworms under simulated soil/field conditions. These studies have recently shown that tefluthrin, a synthetic pyrethroid registered for wireworm control on corn is quite repellent to A. obscurus and L. canus, and lindane is partially repellent to L. canus, but not to A. obscurus. In related work, repellency has also been demonstrated with several natural products (i.e. thymol, citronellal, eugenol and rosemary oil (Waliwitiya et al. 2005). None of the neonicotinoids tested (imidacloprid, thiamethoxam) were found to be repellent.

Discussion

From the studies and collective data discussed above, a number of conclusions can be drawn with respect to how the leading candidate wireworm insecticides (i.e. neonicotinoids, phenyl pyrazols, and synthetic pyrethroids) might actually be affecting wireworm populations in the field, and how efficacy studies might be planned and better interpreted in the future. For example, efficacy trials that only measure reductions in symptoms of wireworm damage (i.e. crop stand and yield as measured in cereal crops, or cosmetic damage as measured in potato crops), are often equated with 'control' (mortality) of wireworm populations. Although this was likely a safer assumption with the older OC, OP and carbamate insecticides (we have evidence in our studies for this statement), equating damage protection with wireworm mortality is tenuous with the newer chemistries, as was shown above (Figs 1-4). Reductions in symptoms of damage might arise if wireworm populations enter prolonged states of non-lethal intoxication (e.g. neonicotinoids), or if populations are repelled long enough to enable crop establishment (e.g. synthetic pyrethroids). One way to better evaluate the efficacy of a candidate insecticide in terms of crop protection versus wireworm population reduction, would be to sample insecticide-treated plots for wireworm populations in the following spring.

References


New approaches to wireworm management in the UK

William E Parker
ADAS, Woodthorne, Wolverhampton, WV6 8TQ, UK.

Abstract: Wireworms, particularly Agriotes obscurus, A. sputator and A. lineatus are becoming an increasing problem for UK farmers, particularly for growers of high value crops such as potato. The lack of effective insecticides for wireworm control means that alternative methods of control are needed to ensure that the risk of wireworm damage is minimised. In the UK, a new project on sustainable long-term management of wireworms is just starting. This will include work on novel approaches to control such as the use of biofumigants (Eruca sativa and Brassica juncea), and novel biocontrol agents such as Metarhizium anisopliae. Work will also be done investigating the interactions between cultivation and insecticide use on the maintenance/decline of wireworm populations in all-arable (no grass) rotations – a particular issue in the UK at present.

Key words: wireworms, Agriotes, integrated control, biofumigants, biocontrol agents

Introduction

Wireworms, the soil-dwelling larvae of click beetles (Agriotes spp., Coleoptera, Elateridae), are widely distributed throughout Europe (Furlan et al., 2002). In the UK, the main species of agronomic importance are Agriotes obscurus, A. lineatus and A. sputator, all of which have similar life cycles and often occur together in the same field. They have the potential to attack a wide range of crops including cereals, sugar beet, carrot and other vegetables (Miles, 1942) and soft fruit, but the most serious damage usually occurs on potato. Potato crops are particularly susceptible to attack as wireworm damage to tubers reduces crop quality rather than gross yield, and even low populations (<100,000 ha⁻¹) can cause an economic level of damage (Parker & Howard, 2001).

In the UK, work between 1999 and 2004 concentrated on evaluating the state of knowledge of wireworm management in relation to potatoes (Parker & Howard, 2001), and in evaluating the recently developed click beetle pheromone trapping system (Furlan et al., 2002, Toth et al., 1998; Toth et al., 2003) as a highly sensitive risk assessment tool to complement existing soil sampling, bait trapping (Parker, 1994; Parker, 1996) and other risk assessment criteria (Parker & Seeney, 1997). This culminated in the trialling of a commercial version of the pheromone trapping system in the U.K. in 2003/04 and its full scale commercial introduction in 2005 (Parker, 2005). However, these improvements in risk assessment systems have actually demonstrated that wireworms are much more widespread in U.K. arable farmland, albeit at lower population levels, than was previously thought. In particular, populations in fields with no long-term history of grass (the traditional high risk situation) are still increasingly troublesome for potato growers. Currently, only two insecticides (ethoprophos and fosthiazate) are registered for use against wireworms on potato in the U.K. Neither are fully effective and there is an urgent need to identify alternative methods of control that consider both strategic (rotational) control and improved levels of tactical (in-crop) control. Such methods also need to be environmentally acceptable and sustainable. A new collaborative project is just beginning in the U.K. that aims to address
these issues. As no data are yet available from the work, this paper reviews the basis of some of the work which will be done in this project.

**Project objectives**

1. To provide a robust method for identifying wireworm larvae.
2. To assess the impact of cultural and rotational factors on the survival of wireworms in all-arable rotations.
3. To investigate the magnitude and timescale of dispersal of click beetles on a landscape scale.
4. To investigate behavioural responses of click beetles to pheromones.
5. To assess the impact of novel biocontrol agents on wireworms and their integration into potato cropping.

**Wireworm identification**

Current, morphological methods of wireworm identification are laborious and require specialist skills. The aim of this work is to develop simple molecular genetic diagnostic tools which will enable accurate identification of larvae. This will improve our ability to determine subtle differences in the ecology of different *Agriotes* instead of assuming that all species behave similarly.

**Impact of cultural factors on wireworm survival in arable rotations**

This work will test the hypothesis that the cultural and agronomic regime applied in an arable field in the years prior to potato cropping influence the maintenance or decline of wireworm population levels, and hence determines the risk of wireworm attack to a subsequent potato crop. The work will use large plots (20 m x 30 m) to compare the effect of different wheat production regimes and a permanent grass control on wireworm populations over two cropping years (06/07 and 07/08). Essentially, wheat production systems will assess the effect on wireworm populations of the interaction between cultivation system (plough vs minimum tillage) and wheat sown either with or without the use of an insecticidal seed treatment (clothianidin). In 2009, potatoes will be grown in the plot areas (i.e. superimposed on the wireworm populations developed during the wheat phase). A range of novel biocontrol treatments developed under Objective 5 will be assessed in the potato phase of the experiment. The work is being done on a commercial arable farm near Cambridge, U.K., that has a history of wireworm damage. One of the challenges of this part of the project is to identify assessment techniques that can identify subtle changes in relatively low wireworm populations over long periods of time.

**Magnitude and timescale of click beetle dispersal in the landscape**

Information arising from mark-recapture studies (e.g. Blackshaw et al., 2006) indicates that adult male movement in response to a pheromone trap is approximately 5 m per week. These trap catch findings, however, are as a result of directional stimulation and only apply to males. Hence they do not define the magnitude of dispersal. The critical factor in deducing the rate of population spread (and hence potential colonisation in a crop from refuges) will be how far the females disperse. Currently, we have no way to realistically undertake mark-recapture studies with females, so we will address this objective through the investigation of gene flow between populations. We will define the maximum dispersal distance, and test the hypothesis that this distance does not differ between the three species. This will help assess the degree of risk presented by adjacent non-arable land such as conservation headlands and beetle banks.
Behavioural responses of click beetles to pheromones

Pheromone traps are highly effective and specific in capturing click beetles (Furlan et al., 2002). Work in the U.K. indicated that there was a useable relationship between pheromone trap catches and residual wireworm populations the following autumn (Parker, 2005). The system is also capable of identifying infested sites where wireworm detection by conventional soil sampling would be difficult. The success of this work led to the commercial introduction of the traps into the UK in 2005. However, improved risk assessment has highlighted the need for a better understanding of adult biology in a landscape context to ensure that pheromone trap catches are interpreted correctly. At present it is assumed that catches of the three wireworm species can be interpreted the same way. Spatio-temporal studies of pheromone trap catches of A. lineatus and A. obscurus in Canada suggest that this may be true at the landscape scale (Blackshaw & Vernon, 2006), but not at the field scale. Thus it is important to tease out interspecific differences in order to better interpret trap catches, particularly as the evidence suggests that relationships between wireworm populations in the soil and pheromone trap catches are not always reliable, due partly to inter-field variations in the life-stage structure of the wireworm population, and partly to interspecific differences in the response to pheromone traps of different Agriotes species.

Impact of novel biocontrol agents and their integration into management strategies

This work will identify whether novel approaches to biocontrol of wireworms can reduce the need for conventional soil insecticides through direct replacement or reduction in applied insecticide dose rates. Initial work will be done at a bioassay (Year 1) and semi-field (Year 2) scale. Years 3 will test individual components in the field, and Year 4 will investigate full integration into potato production. Work will concentrate on the practical efficacy and use of microbiocntrol agents such as Metarhizium anisopliae and entomopathogenic nematodes, as well as work on mustard crops and mustard meals that can be used as biofumigants. There has been some limited initial work on the use of biofumigants for wireworm management (Furlan et al., 2004), but none that relates directly to UK Agriotes species, climate or production methods. The mustards under test in the U.K. are Brassica juncea and Eruca sativa. A defatted mustard meal product derived from Brassica carinata is also being tested. We also hope to draw on work on M. anisopliae that is on-going in Canada (e.g. Kabaluk et al., 2005) and in Europe (e.g. Ghormade et al., 2007).

Acknowledgements

On-going work is funded under SA-LINK project LK0982, sponsored by the Department for Environment, Food & Rural Affairs (Defra), the British Potato Council, Solanum Ltd, Greenvale AP, Babraham Farms, Becker Underwood Ltd, Syngenta Crop Protection, Bayer CropScience and J Sainsbury plc and Farmcare.

References


Comparison of three different bait trap types for wireworms (Coleoptera: Elateridae) in arable crops

Nina Brunner, Eva-Maria Grünbacher, Bernhard Kromp
Bio Forschung Austria, Rinnböckstraße 15 1110 Wien, Austria

Abstract: Wireworms are abundant dwellers of arable soils, causing economically severe damage on potatoes, maize and other arable crops (e.g. sunflower, rape, asparagus, lettuce, onion). Since pesticide application for direct control is forbidden in organic farming, assessment systems for detecting wireworm infestation levels and forecasting damage thresholds are urgently needed. However, the basis of any wireworm risk assessment is a proper sampling technique for recording wireworm occurrence and abundance in the soil. In earlier studies comparing different baited and unbaited wireworm sampling methods, cereal baited traps were found to be most effective. The present study was carried out from May to September 2005 in two potato fields and one maize field in Vienna/Austria. It aimed at finding out the most effective and time-efficient method among three different cereal (maize/wheat mixture) baited trap types: I. plate-trap (Jossi & Bigler 1997), II. pot-trap (adapted from Kirifman & Armon 1986), III. mesh-bag (Horton & Landolt 2002, adapted by Brunner et al. 2005). In each field, 20 traps of each type (reduced to 6 traps from July onwards) were set along two transects (distance between sampling points: 7 m). Additionally, at each sampling point, one potato plant was inspected for wireworm damage before harvest. The traps were changed every 2 to 4 weeks. A soil core of 15 cm in diameter was taken around the traps to get hold of wireworms outside the containers. The bait mixture and the surrounding soil were hand-sorted separately for larvae. Altogether, 1,988 wireworms were trapped. Considering the total wireworms caught inside and outside the bait traps, the three trap types did not differ significantly from each other (Kruskal-Wallis-Test). However, considering only the larvae caught inside the traps, pot-traps contained significantly more larvae (3.3 inds/trap) than plate-traps (2.4 inds/trap; p = 0.038) and mesh-bags (1.2 inds/trap; p ≤ 0.001; U-Test). As to the practical implementation in wireworm field-studies, we consider the pot-trapping method advantageous, since 75% of baited larvae were found inside the pots, compared to 63% inside the plates and only 53% inside the mesh-bags. Therefore, bait-trapping with the pot method can be done without the time-consuming evaluation of the surrounding soil cores.

Keywords: wireworms, Elateridae, baiting, sampling, pot-trap, plate-trap, mesh-bag, risk assessment

Introduction

Wireworms are abundant dwellers of arable soils, causing economically severe damage on potatoes, maize and other arable crops (e.g. sunflower, rape, asparagus, lettuce, onion). Since pesticide application for direct control is forbidden in organic farming, assessment systems for detecting wireworm infestation levels and forecasting damage thresholds are urgently needed. Knowing the risk of an infestation or even the level of damage in advance, farmers could react in time and possibly avoid planting susceptible crops into infested fields. However, the basis of any wireworm risk assessment is a proper sampling technique for recording wireworm occurrence and abundance in the soil. In earlier studies comparing different baited and unbaited wireworm sampling methods (e.g. Brunner et al. 2005, Lefko et al. 1998, Parker 1994), cereal baited traps were found out to be most effective. The present
study aimed at finding out the most effective and time-efficient method among three different cereal (maize/wheat mixture) baited trap types.

Material and methods

Sampling sites and trap types
The sampling sites, two potato fields and one maize field, located in Vienna/Austria, were chosen due to wireworm infestations in earlier years. From May to September 2005 the efficacy of the following three bait trap types was compared:

I. plate-trap (Jossi & Bigler 1997)
A plastic plate of 9 cm in diameter with 5 holes in it (about 3 mm diameter) and a rim of 2 cm height, dug in 10 cm depth.

II. pot-trap (adapted from Kirfman & Armon 1986)
A plastic flower pot with a capacity of 500 ml dug in 15 cm depth with a plastic plate (12 cm in diameter) placed 3 cm above and covered with soil.

III. mesh-bag (Horton & Landolt 2002, adapted by Brunner et al. 2005)
A mesh-bag, 20 x 20 cm, with a net width of 3 cm dug in 10 cm depth.

The bait mixture was the same for the three types: Wheat and maize grains were soaked with water for about 12 hours. The water-soaked wheat and maize grains (about 30 ml each) were mixed with about 100 ml of Vermiculite. After filling the traps with the mix, they were watered again.

Sampling design
Per field, 20 traps of each type were set in two plots of 10 sampling points each. The plots were chosen due to different site parameters as follows: near the field margin (headland, 2-years of alfalfa as a previous crop) and inside the field (shallow soil located on gravel, winter wheat as a previous crop) in the maize field, the cultivars Princess and Nicola (located on dry, deep chernozem) in one potato field (P1) and a slight depression and a slight elevation shaped by the undulation of gravel in the soil profile in the other potato field (P2; grey alluvial soil, cultivar Agata).

At each of the 20 sampling points, the three different trap types were placed in adjacent rows in the potato fields, and with one row between them in the maize field. The distance between sampling points was 7m until the number of sampling points was reduced from 20 to 6 after two sampling periods. After that the distance between sampling points was varied between 14 and 21m. Bait traps were changed every 2 to 4 weeks. A soil core of 15 cm in diameter was taken around the traps to get hold of wireworms outside of the bait. The bait mixture and surrounding soil were hand-sorted for larvae.

Additionally, in the end of the sampling season just before harvest, at each sampling point one potato plant was inspected for wireworm damage. In the potato field P1 the cultivar Princess was inspected on 26 August, the cultivar Nicola on 12 September. The cultivar Agata in the other potato field (P2) was inspected on 29 July. For the inspection of maize plants, on 22 June in each of the two plots, 4 sections of 7 m length and a width of one planting row were chosen. The plants per section were counted and those with inhibited growth were inspected for wireworm damage on the stem base.

Results and discussion

A total of 1,988 wireworms, to a large extent of the genus *Agriotes*, were caught during the sampling season. Comparing the three fields (Table1), the highest number of wireworms, a
Table 1. Number of traps and total number of baited larvae per sampling date and site as well as the mean number of baited larvae per trap ± standard deviation per sampling date and site, in 2005. Catches of all trap-types were summed up. M = maize field, P = potato field (1 and 2), “inside” = inside the traps, all three fields located in Vienna.

<table>
<thead>
<tr>
<th>sampling site</th>
<th>sampling date</th>
<th>number of traps per type</th>
<th>total number of baited larvae</th>
<th>total number of baited larvae inside</th>
<th>mean nr. of larvae/trap ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>03 June 2005</td>
<td>20</td>
<td>572</td>
<td>216</td>
<td>9.5 ± 7.3</td>
</tr>
<tr>
<td>M</td>
<td>22 June 2005</td>
<td>20</td>
<td>425</td>
<td>268</td>
<td>7.2 ± 4.9</td>
</tr>
<tr>
<td>M</td>
<td>04 July 2005</td>
<td>6</td>
<td>72</td>
<td>21</td>
<td>4 ± 3.1</td>
</tr>
<tr>
<td>M</td>
<td>26 August 2005</td>
<td>6</td>
<td>40</td>
<td>26</td>
<td>2.2 ± 1.4</td>
</tr>
<tr>
<td>M</td>
<td>12 September</td>
<td>6</td>
<td>47</td>
<td>31</td>
<td>2.6 ± 2.7</td>
</tr>
<tr>
<td><strong>total</strong></td>
<td></td>
<td></td>
<td><strong>1,156</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>15 June 2005</td>
<td>20</td>
<td>316</td>
<td>208</td>
<td>5.3 ± 5.5</td>
</tr>
<tr>
<td>P1</td>
<td>04 July 2005</td>
<td>20</td>
<td>300</td>
<td>190</td>
<td>5.0 ± 4.6</td>
</tr>
<tr>
<td>P1</td>
<td>29 July 2005</td>
<td>6</td>
<td>45</td>
<td>32</td>
<td>2.5 ± 2.2</td>
</tr>
<tr>
<td>P1</td>
<td>26 August 2005</td>
<td>6</td>
<td>42</td>
<td>29</td>
<td>2.5 ± 2.2</td>
</tr>
<tr>
<td>P1</td>
<td>12 September</td>
<td>10</td>
<td>28</td>
<td>16</td>
<td>0.9 ± 3.3</td>
</tr>
<tr>
<td><strong>total</strong></td>
<td></td>
<td></td>
<td><strong>731</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>14 June 2005</td>
<td>20</td>
<td>42</td>
<td>33</td>
<td>0.7 ± 0.9</td>
</tr>
<tr>
<td>P2</td>
<td>04 July 2005</td>
<td>20</td>
<td>45</td>
<td>36</td>
<td>0.8 ± 1.2</td>
</tr>
<tr>
<td>P2</td>
<td>29 July 2005</td>
<td>6</td>
<td>14</td>
<td>10</td>
<td>0.8 ± 1.0</td>
</tr>
<tr>
<td><strong>total</strong></td>
<td></td>
<td></td>
<td><strong>101</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

total of 1,156, was caught in the maize field, followed by the potato field P1 with a total of 731 larvae baited.

Wireworms were obtained in every sampling period throughout the season. Catches were highest in June and the start of July and decreasing towards September. The total catches were similar for the three trap types (Table 2). However, considering only the larvae caught inside the traps, the three types did differ significantly from each other (Kruskal-Wallis-Test; \( \chi^2 = 27.2; p \leq 0.001 \)). Pot-traps contained significantly more larvae (3.3 inds/trap) than plate-traps (2.4 inds/trap; \( Z = -2.071; p = 0.038 \); U-Test) and mesh-bags (1.2 inds/trap; \( Z = -5.245, p \leq 0.001 \); U-Test). Also the maximum number of larvae per trap was highest (32) in the pot-trap, followed by the mesh-bag (25) and the plate-trap (23).
Table 2. Number of baited larvae per trap-type in two potato fields and one maize field in Vienna from June to September 2005 (mean ± standard deviation). n = 166 traps per type; “inside” = inside the trap, “outside” = in the soil core around the trap, “total” = inside and around the trap.

<table>
<thead>
<tr>
<th>larvae per trap</th>
<th>pot- trap</th>
<th>plate - trap</th>
<th>mesh - bag</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean number inside</td>
<td>3.3 ± 4.2</td>
<td>2.4 ± 3.0</td>
<td>1.2 ± 1.9</td>
</tr>
<tr>
<td>mean number outside</td>
<td>0.8 ± 1.7</td>
<td>1.8 ± 2.8</td>
<td>2.5 ± 4.0</td>
</tr>
<tr>
<td>total number</td>
<td>4.1 ± 5.3</td>
<td>4.1 ± 5.2</td>
<td>3.8 ± 5.0</td>
</tr>
<tr>
<td>maximum number inside</td>
<td>22</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>maximum number outside</td>
<td>12</td>
<td>21</td>
<td>22</td>
</tr>
<tr>
<td>maximum number total</td>
<td>32</td>
<td>23</td>
<td>25</td>
</tr>
<tr>
<td>percentage of traps with at least one larva inside</td>
<td>75 %</td>
<td>63 %</td>
<td>53 %</td>
</tr>
<tr>
<td>percentage of traps with at least one larva total</td>
<td>80 %</td>
<td>71 %</td>
<td>72 %</td>
</tr>
</tbody>
</table>

Since 75% of the pot-trap-baited larvae were found inside the containers, sampling might be done without taking a surrounding soil core without much loss of information, which saves a lot of working time. Furthermore, the given volume of the pot trap enhances comparability.

Wireworm damage

The extent of damage as well as the number of baited wireworms differed not only between, but also within the individual fields. Apart from wireworm incidence, many other factors can influence the damage level. The degree of infestation may partially be influenced by the soil conditions (Strickland et al., 1962 cited in Parker& Howard, 2001). In the present study, considering the different plots in each field, highest catches were obtained in soil located on gravel lenses in the maize field as well as in the potato field P2 (Figure 1).

As to the relation of wireworm numbers and crop damage, each of the three trap types baited more larvae on the more heavily infested plots (Figure 1). In the “slight elevation”- plot in the potato field P2, the percentage of damaged potatoes was as high as 32 % with an average of 1.1 larvae caught inside the pot traps. In the “slight depression”- plot an average of 0.5 larvae per pot-trap and a crop damage of 6% were observed.
Figure 1. Mean number ± standard deviation of baited larvae per trap type in different plots of a maize field and two potato fields in Vienna in the sampling season 2005 and the percentage of damaged maize plants/ potato tubers. M = potato field; P1, P2 = potato field.

As to the practical implementation of wireworm bait traps, the patchiness of wireworm occurrence hampers the development of proper sampling designs that, as we consider, will have to be adapted individually to respective field conditions. For the development of a risk assessment system, it will be important to study the influence of environmental parameters on the occurrence and abundance of wireworms in the soil.

References


and diversity in Iowa conservation reserve environments. Environmental Entomology, 27: 312-317


Practical Dutch experience introducing a monitoring system of click beetles by pheromone traps.

Klaas van Rozen¹, Albert Ester¹, Ton Hendrickx²
¹ Applied Plant Research (PPO-AGV) Wageningen University and Research, P.O.Box 430, 8200 AK Lelystad, The Netherlands. ² Coöperatieve Zuidelijke Aan- en Verkoopvereniging u.a. (CZAV), P.O. Box 402, 4460 AV Goes, The Netherlands

Abstract: A new supervised control system against wireworms is introduced in the Dutch agriculture, using a click beetle trap (Kniporkit) for monitoring the adult beetles, followed by a pyrethroid control at a defined time point if necessary. Therefore, the beetles instead of the wireworms are controlled. The Kniptorkit is a trap equipped with a sexual lure (pheromone), attracting male click beetles. Attractively for farmers who have already experience with wireworm problems on certain fields. Practical experience started in 2004 with 56 growers using this trap mainly in grass seed and wheat crops having potatoes in their crop rotation. In 2005 the number of growers increased to 105 participants using this monitoring and control system. Captures between regions and between several fields can differ strongly. Differences are caused by natural presence in the area, field history and the host plants for egg deposition in which the traps are placed. Click beetles deposit eggs mainly in monocotyle crops. It is important to observe the presence of the click beetles per field, since research has shown that wireworms are not found on every field. This may limit the use of pesticides, an initial aim next to provide growers good alternative controlling wireworms.

Key-words: Agriotes lineatus, A. obscurus, sex pheromones, monitoring, control, Kniptorkit.

Introduction

In The Netherlands Agriotes lineatus and A. obscurus are the most important click beetles causing larval feeding damage to potatoes. Larvae of click beetles are generally known as wireworms. Potato crops are particularly susceptible to attack as wireworm damage to tubers reduces crop quality rather than yield. Even low populations (< 100,000 per ha) can cause economic damage (Parker & Howard, 2001). Chemical control of Agriotes spp. is historically based on the control of the wireworms. Avoiding haphazardly control, methods of predicting wireworm densities do exist for decades. Although soil sampling (Salt & Hollick, 1944) or baiting techniques (Parker, 1996) have been developed, these techniques may lack practical implementation due to laborious factors or factors concerning insufficient threshold interpretation. In Dutch potato cropping systems, it is recommended to assess the soil on wireworm presence or absence just prior to planting. The bait system used provides the assessment of the fields on wireworms using halved potatoes, which are dug into the soil. After two weeks the halved potatoes are assessed on presence or absence of wireworms. The threshold is discussable, as one wireworm is sufficient for advising a soil treatment and absence of wireworms are not a security for a wireworm damage free harvest. Another disadvantage is the timing of the wireworm assessment. Planting the potatoes early in the season is in general during periods of lower temperatures, at which stage the wireworms have a lack of activity or may be dwelling deeper in the soil. This means that the bait-sampling technique may miss wireworms due to inactivity, a requirement for baiting application.
Concerning the chemical control of the soil dwelling wireworms in The Netherlands, availability of legally allowed active ingredients against this pest is reduced to one, namely ethoprofos. A need for alternative methods controlling wireworms was urged by the Dutch farmer’s organization late last century. The main focus became the adult click beetles, starting research to monitor Agriotes spp. with sex pheromones in the Dutch polders (Ester & van Rozen, 2001). A. lineatus and A. obscurus were most abundant in the arable fields, out of five species tested for attraction with specific sex pheromones (Plant Research International; www.pri.wur.nl). Male A. lineatus and A. obscurus were highly specific attracted by the female sex pheromones. The sex pheromone mixtures gave a good indication of presence and flight peaks of these populations (Ester et al., 2002). Field trials followed-up in the years after, aiming the application of a pyrethroid insecticide at a specific time point of the click beetle flight, monitored by adult beetle traps (Kniptorkit). After chemical application (just after a clear peak of the flight) the numbers of caught adult Agriotes spp. declined rapidly, assessed by the traps with sex pheromones as well (Ester et al., 2004; Ester & van Rozen, 2005). Starting in 2004, two farmers cooperation’s have introduced the system, both in their own way. The development and progress of this supervised control system of A. lineatus and A. obscurus is discussed. The aim of the supervised control system is reducing wireworms to an extent of non-economical damage of potatoes in the field. Naturally, damage as low as possible is a serious issue, as contamination of potatoes with wireworm feeding damage does take significant more labour efforts to get a saleable product.

System overview

The click beetle supervised control system is advised to farmers which have a historical nuisance with Agriotes spp. larvae in the field. Using female sex pheromones attracting the males into the traps may indicate to a certain extent the wireworm densities in the next years. If male Agriotes spp. are showing a peak catch during the season, what means mating activity prior to eggs laying by the females, control will be conducted in time. In this view, when controlling the male click beetles it is assumed that female click beetles are controlled as well. Considered the amounts of eggs laid by a Agriotes spp. (approximately 78 eggs per female; Furlan, 1996; unpublished data), this indicates a huge population impact on next generations of Agriotes spp. Nevertheless, in natural conditions a considerable reduced level of survival of eggs and early instar larvae may have a decreasing impact on the population.

Progress and discussion

Practical experience in 2004 showed differences in captures between different regions and different fields within regions. Most probably differences may occur due to natural presence in the area, field history, and the current crop in where the traps are established. Farmers in the southwest of The Netherlands were most interested in the click beetle supervised system. In 2004 57 farmers participated in a semi commercial-development promoting activity initiated by a farmers cooperation. Traps were placed in 60 fields with an approximate area of 400 ha. This area is characteristic with a rotation of grass seed – winter wheat – potatoes by farmers. The monocotyl crops do act as a good host for egg laying Agriotes spp. females, increase of a population may well be considered in such a rotation. High populations of Agriotes spp. can be present in grass seed crops, cereal crops or even weedy fields. In 2004 traps with sex pheromones were placed on approximately May 1, as recommended. It is necessary to assess the click beetle abundance from the first emergence on, in order to be able to detect a regular peak. In the Dutch Flevopolders click beetles emerged around the first of
May, but this was too late for the southwest of The Netherlands. Activity in southwest may be starting earlier as the temperatures are generally higher than up north. For 2005 placement of the traps was recommended to start before May 1. Each five ha of monitored crop received four traps, two baited with *A. lineatus* and two traps baited with *A. obscurus* specific lures. Each 5 ha more two extra traps were recommended. Distance between the traps was 20 m, placed as much as possible in the middle of the fields. Trap assessment was advised twice a week, which meant emptying the traps, counting the adult *Agriotes* spp. and writing the total numbers per trap into a time-table. After appearance of a peak, i.e. right after counts started to decline, a pyrethroid should be applied. As soon as possible farmers were advised to control the click beetles in the evening hours in fertile weather conditions, for two reasons. Firstly, click beetles seemed to be more active in the trials during the evening. *Agriotes* spp. are negative photo-tactic (Blunck & Muehlmann, 1954), and the beetles are more active during the night in humid weather (Edwards & Heath, 1964). Secondly, as general accepted, pyrethroids may be effective more if the product is not exposed to direct sun light which breaks down the chemical quickly. In practice, when the grass seed crop is subsided, the pyrethroid should be applied with more water up to 500 l per ha to get the active ingredient to the base of the crop. Continuing the monitoring of the click beetle population was highly advised and conducted by the farmers. The effect of the pyrethroids was not assessed the first trapping moment after the application, but the second (Figures 1 and 2). Both treatments gave certainty about the effect of the control activity of different pyrethroids for the farmer, if desirable a second treatment could be considered. It is shown that the captures between regions and between several fields can differ strongly. Differences may be caused by natural presence in the area, field history and the host plants for egg deposition in which the traps are placed.

Figure 1. Practical results of monitoring adult *Agriotes* spp. and control by 10 g a.i. lambda-cyhalothrin per ha, applied in the evening.
Grass seed (M = timing treatment on 17 May, Nieuwe Tonge)

Data of monitoring (2004)

Figure 2. Practical results of monitoring adult *Agriotes* spp. and control by 7.5 g a.i. deltamethrin per ha, applied in the evening

In 2005 the supervised control system was more commercialized and in the southwest of The Netherlands 105 farmers utilised this control strategy of the click beetles. This included fields of an area of approximately 700 ha. Ninety starter’s packages were taken, including four traps and eight dispensers, sufficient for five ha monitoring. Additional 59 more traps were bought and a total of 471 dispensers, for each 5 ha more per field. The main crop monitored was grass seed, and winter wheat was the second main crop followed by some other cereals and alfalfa. It was advised to start trapping before May 1, based on the experiences gained in the previous year. The system was more standardised due to the input of farmers, suppliers and scientists. The main motivation was to make trapping and counting less laborious. As a consequence, the trap distance from the field border was appointed to a minimum 15 m and the frequency of trap assessment was reduced to once a week. The decision for pyrethroid applications remained the same i.e. treatments were performed right after counts started to decline.

During the season of 2006 approximately 130 farmers with an estimated area of 900 ha continued the monitoring and control system in the southwest of The Netherlands. Trapping was recommended to be started April 20. The main difference compared to the year of 2005 was the implementation of a database on a website. Anticipating farmers were able to log in and submit their data directly. The system also provided the farmer with an immediate recommendation whether a treatment should be applied or not. The threshold of numbers of click beetles was increased. Treatments were only recommended after a peak, but with a minimum of 10 beetles per trap average. In previous years farmers experienced unwanted catches of other beetles including, spiders and mice. At least the mice problem was solved, using a specific crucifix preventing these animals entering the trap.

Two suppliers of traps and lures introduced different systems of service and recommendation to the farmer. The first supplier introduced a data base on the website, which allowed direct access for the farmer. It allows the farmer to register weekly counting’s of the numbers of caught beetles and it provides recommendations whether treatments are suggested or not. The second supplier is more active up north in The Netherlands. Its activity includes a complete service of trap placement, assessing weekly the adult beetle captures and farmers are informed weekly if a treatment is required. Farmers just need to apply the insecticide treatment.

In perspective, the use of adult beetle trapping may well improve the risk assessment for wireworm infestations. Adult control is an alternative to the wireworm control, although the
relation to a reduced wireworm population has not been proven directly. The numbers of decreased click beetles per trap is assessed after treatment. Significant fewer beetles are trapped after treatment (Ester & van Rozen, 2005), which indicates a decrease of the population in the field. Adult Elateridae do not migrate, and any movement is therefore likely to result in local dispersal only, probably largely by walking, because some species (e.g. *A. obscurus*) do not seem to fly readily (Parker & Howard, 2001). As a consequence the click beetle population may be reduced to a small non harming level per field after chemical control. The monitoring system of adult *Agriotes* spp. may also lead to other practical implementations in arable farming. Based on numbers of click beetles during the previous year, farmers may consider a change of crop rotation using a less vulnerable crop. If practical, which seems to be possible for crops like grass seed or cereals as the harvest occurs in summer months, soil cultivation may decrease wireworm populations. Especially on warm and dry days, eggs and early instars of *Agriotes* spp. may be quite susceptible to cultivation methods which may lead to starvation. Repeated disturbance of the soil decreases wireworm populations (Seal et al., 1992). In addition, knowing the adult *Agriotes* spp. presence and density per field in a previous year, a soil treatment against wireworms may be recommended before growing vulnerable crops like potatoes. Determination of the presence of adult click beetles is more convenient than sampling for the wireworms. If the abundance of the click beetle population in a field is known, sufficient time is available for control action. Soil sampling just prior to potato planting only allows for soil insecticide application due to lack of time searching for an alternative. The click beetle supervised control system aimed for application in an arable crop rotation to control the so called ‘arable wireworm’, named by Parker & Howard (2001). But this system may be used as well as a monitoring tool for managing the wireworms in crops after long-term grassland. The best indicator of wireworm presence or absence is the duration of grassland in the cropping history of individual fields (Parker & Howard, 2001). Practical biological control against adult *Agriotes* spp. has not been thoroughly assessed yet, but it may have good potential for click beetle control. Modified traps might be used to inject large numbers of adult Japanese beetles with fungi (e.g., *Metarhizium anisopliae*) or other entomopathogens, with subsequent autodissemination into larval habitats (Potter & Held, 2002). In Germany sex pheromone use may be well considered for organic farming systems (Böhm & Krause, 2006). As arable farming systems have relatively small experiences with assessing common pest abundances compared to horticulture, especially greenhouse systems, a practical tool is available now for farmers.

References


New sex attractant for *Agriotes proximus*: similarities in pheromonal communication with *A. lineatus* (Coleoptera: Elateridae)

Miklós Tóth1 Lorenzo Furlan2, Amália Xavier3, József Vuts1, Mitko Subchev4, Teodora Toshova4, István Szarukán5, Venyamin Yatsynin6

1 Plant Protection Institute, HAS, Budapest, Herman O. u. 15, H-1022 Hungary; 2 Department of Agronomy, Entomology, Padova University, Agripolis, Via Romea 16, Legnaro, I-35020 Italy; 3DRAEDM, Porto, Rua da Resauracao 336, P-4050-501 Portugal; 4 Zoology Institute, Bulgarian Academy of Science, Sofia, blvd Car Osvoboditel 1, BG-1000, Bulgaria; 5 Agricultural University, Debrecen, POB 58, H-4001, Hungary; 6 Krasnodarskiy NIISKh im. P.P. Lukyanenko, Krasnodar 12, Russia, 350012

Abstract: When testing traps baited with a blend of geranyl octanoate and geranyl butanoate (the pheromone components of *Agriotes lineatus*, Coleoptera, Elateridae) in Portugal, large numbers of the closely related *A. proximus* were captured. This was highly surprising, as in the literature two completely different components, (E,E)-farnesyl acetate and neryl isovalerate were previously described as pheromone components of *A. proximus*.

Later tests conducted in several countries of Western and Central Europe revealed that the butanoate on its own showed low but significant attraction for *A. proximus*, while the same was true for the octanoate and for *A. lineatus*. However, largest catches were observed with a blend of the optimal 1:1 ratio in both species. No *A. proximus* catches were recorded in traps baited with the blend of (E,E)-farnesyl acetate and neryl isovalerate at any of the test sites.

In electroantennography (EAG), male antennae of both *A. proximus* and *A. lineatus* responded better to geranyl butanoate than to geranyl octanoate, suggesting that there were no differences between the two species at the sensory level either.

In conclusion, we found remarkable similarities in the two species as far as pheromone perception (EAG responses) and field responses to pheromone components were concerned. As for pheromone composition when extracted from females, in *A. lineatus* the major component was found to be geranyl octanoate by earlier studies, while geranyl butanoate was detected only in traces. Pheromone extraction of *A. proximus* populations is planned in the future. The two species are considered clearly separated due to mainly one constant morphological difference but there is no study demonstrating biological separation. Genetic or classical biological studies would be needed to clear up this issue.

From the practical viewpoint, it appears that the 1:1 blend of geranyl octanoate and geranyl butanoate can be used as a bait in pheromone traps for the detection and monitoring of both *A. lineatus* and *A. proximus* in Europe.

Key words: pheromone trap, geranyl butanoate, geranyl octanoate, *Agriotes proximus*, *Agriotes lineatus* Coleoptera, Elateridae

Introduction

In the course of our Europe-wide testing of click beetle pheromones, to our surprise traps baited with the pheromone bait for *Agriotes lineatus* L. (Coleoptera, Elateridae) in Portugal regularly captured specimens of the closely related *A. proximus* Schwarz (Tóth & Furlan, 2005, and paper in this issue). The bait for *A. lineatus* consisted of a blend of geranyl
butanoate and geranyl octanoate (Tóth et al., 2003). The catches of *A. proximus* were quite unexpected, because in the literature two completely different components, \((E,E)\)-farnesyl acetate and neryl isovalerate were previously described as pheromone components of *A. proximus* (Yatsynin et al., 1980, 1996).

Intrigued by this controversy we set out to study the responsiveness of *A. proximus* and *A. lineatus* to the above compounds, with the final aim of defining a practically useable pheromone bait composition for *A. proximus* populations in Western and Central Europe.

**Material and methods**

Field tests were conducted at several sites in Bulgaria, Hungary, Portugal, and Russia (Krasnodar region). Trapping tests were conducted according to internationally accepted methods for such assays. For details of single tests please refer to the Figure legends or the respective reference cited.

Synthetic geranyl butanoate and octanoate were purchased from Bedoukian Inc. (Danbury, USA) and were >99% pure as verified by GC. The compounds were formulated by the usual methods in polyethylene dispensers (for details see for example Tóth et al., 2003). In the trapping tests YF traps (Furlan et al., 2004; produced by RO-SA Micromecanica S.R.L., San Dona di Piave, VE, Italy) were used.

For recording electroantennograms (EAG) a stainless steel tube (teflon coated inside) with a constant humidified airflow of ca 0.7 l/min was set up. An antenna freshly amputated at the base from a living adult male click beetle was mounted between two glass capillaries containing 0.1 normal KCl solution, and the mounted antenna was placed at ca. 3 mm distance from the outcoming airflow. One of the electrodes was grounded while the other was connected to a high impedance DC amplifier (AM-02, Syntech, Hilversum, The Netherlands). Test compounds (10 \(\mu\)g ea.) were administered in hexane solutions to a 10 x 10 mm piece of filter paper inside a Pasteur pipette. Stimuli consisted of pushing 1 ml of air through the Pasteur pipette into the airstream flowing towards the antenna. Response amplitudes were normalized against the means of responses to \((E,E)\)-farnesyl butanoate (eliciting medium high responses from antennae), which was tested before and after the test compounds. Stimuli were administered at ca 20-30 sec intervals. Experimental insects were collected from the field in Portugal (*A. proximus*) or in Hungary (*A. lineatus*).

**Results and discussion**

**Field activity of compounds**

In testing a range of ratios of the blend of geranyl butanoate and geranyl octanoate and the single components, in Portugal and Bulgaria the largest numbers of *A. proximus* were caught by the 1:1 mixture. Catches of *A. lineatus* in Hungary and Russia (Krasnodar region) showed the same tendency (results of these tests will be published in detail elsewhere). In traps with the single components, low but significant catches of *A. proximus* were recorded in traps with geranyl butanoate, while low catches of *A. lineatus* were observed in traps with geranyl octanoate; however, numbers caught with the single components were far smaller than those with the blend of the two compounds. No *A. proximus* catches were recorded in traps baited with the blend of \((E,E)\)-farnesyl acetate and neryl isovalerate at any of the test sites.

The importance of the presence of both compounds for *A. proximus* was clearly confirmed in a dose test, where at all dose levels tested the mixture of compounds caught far more than geranyl butanoate alone (Figure 1). A slight tendency of increase was observed in traps baited with a dose of 20 to 200 mg of the mixture, but catches were not significantly
different, suggesting that the optimal dose may fall within this range in *A. proximus* (Figure 1).

**Figure 1.** Catches of *A. proximus* in traps baited with different doses of geranyl butanoate and its blend with geranyl octanoate. Columns with same letter are not significantly different at P<0.05 by ANOVA, Games Howell.

Based on the above results, practical application trials with the geranyl butanoate / octanoate blend were successful in detecting the first appearance and in monitoring the flight dynamics of *A. proximus* in both Portugal and Bulgaria (Figure 2).

**Figure 2.** Seasonal distribution of catches of *A. proximus* in traps baited with the blend of geranyl butanoate and geranyl octanoate.
Electroantennograms
In EAG studies male antennae of both *A. proximus* and *A. lineatus* responded better to geranyl butanoate than to geranyl octanoate, and also the response spectra of the two species to several pheromone components of *Agriotes* spp. showed a very similar tendency of responses (Figure 3), suggesting that there were no differences between the two species at the sensory level. It is notable that geranyl propionate yielded the highest antennal responses in the two species. The importance of this phenomenon should be the subject of future studies. We have not tested this compound in field trapping tests.

![Figure 3. EAG responses of *A. proximus* and *A. lineatus* males to pheromone components of *Agriotes* spp. Responses were normalized against responses to the standard (E,E)-farnesyl butyrate. From each species 30 antennae were tested.](image)

Female-produced pheromone composition
We have as yet no information on pheromone composition of *A. proximus* females. The ratio of geranyl butanoate necessary in field tests for highest catches in *A. lineatus* was a bit surprising, since in previous studies this compound was detected only in traces in female pheromone gland extracts (Figure 4; Borg-Karlson et al., 1988, Siirde et al., 1993, Yatsynin et al., 1996, Tóth et al., 2003). A probable explanation could be that components extracted by direct solvent extraction of pheromone glands may not reflect exactly the ratio of components emitted into the air by a calling female. In the future, analysis of collection of volatiles should be performed to test this hypothesis. Pheromone volatile collection of *A. lineatus* and *A. proximus* populations is planned.
Figure 4. Gas chromatographic analysis of pheromone gland extract of female A. lineatus. (Hewlett-Packard 5890, SP 2340 fused silica 30 m x 0.32 mm id; 0.20 um; carrier He; 60°C 1 min; 10°C/min to 100°C; 5°C/min to 220°C; 40 min. Data from Tóth et al., 2003).

**Conclusions**

In the present studies we defined the blend of geranyl butanoate and geranyl octanoate as a new sex attractant for A. proximus. This composition was clearly more powerful than the composition previously described in the literature. The same blend was the most active combination also for A. lineatus. The only slight difference between the two species in field attractancy was that A. proximus showed a rather low but existing response to the butanoate, while A. lineatus to the octanoate. Antennae of the two species did not show any difference in sensitivity to a range of pheromone components from other Agriotes spp.

The two species are considered clearly separated due to mainly one constant morphological difference but there is no study demonstrating biological separation. Genetic or classical biological studies would be needed to clear up this issue.

From the practical point of view, it appears that the 1:1 blend of geranyl octanoate and geranyl butanoate can be used as a bait in pheromone traps for the detection and monitoring of both A. lineatus and A. proximus in Europe. Although in most cases species show specific pheromone compositions, the case of having identical pheromone components in click beetles is not unprecedented. A. sordidus Illiger and A. rufipalpis Brullé both use geranyl hexanoate in their sex pheromones (Furlan et al., 2004, Tóth et al., 2002, 2003). It is notable that neither in the pair A. sordidus / A. rufipalpis, nor in A. proximus / A. lineatus have we yet found a site where both members of the pair would be present in high populations; always one of the species dominated (pls refer to Tóth & Furlan, 2005, and paper in this issue).

**Acknowledgements**

This research was partially supported by grant NKFP 4/012/2004 OM of the Hungarian Ministry of Education, and by grant 1201/2002 from the Bulgarian National Scientific Fund.
References


Approaches to wireworm control in organic potato production

Daniel Neuhoff¹, Christiana Christen², Andreas Paffrath², Ute Schepl²

¹ Institute of Organic Agriculture, University of Bonn, Katzenburgweg 3, D-53115 Bonn, Germany; ² Agricultural Chamber NRW, Dep. Ecological Agriculture and Horticulture, Gartenstr. 11, D-50765 Köln-Auweiler, Germany

Abstract: Tuber damages caused by wireworms can result in significant economic losses in organic potato production. Since no synthetic insecticides are allowed in Organic Agriculture, wireworm control has to be managed by agronomic approaches. In 2005 organic potato field trials were carried out on 3 sites in Southern North Rhine-Westphalia, targeted at assessing the impact of variety choice (2 - 6 varieties) and harvest date (3 - 5 dates) on wireworm damage of potato tubers. Tuber yield and quality were recorded and submitted to ANOVA. Variety choice had a significant effect on tuber losses caused by wireworms. At the Auweiler site the highest losses were noted in cv. Princess (27.0%) and the lowest in Nicola (12.7%). Bringing forward tuber lifting dates to the middle of August resulted in significantly lower wireworm induced tuber losses compared with middle of September. Early harvesting can be recommended if tuber skin is sufficiently suberised and if cooling facilities are available for the tubers.

Key words: wireworms, potatoes, cultivars choice, tuber harvest date

Introduction

Potato tuber injuries caused by wireworms often result in significant losses of marketable yields. According to a survey made by Keiser et al. (2005) wireworm damages of potato tubers are higher in fields under organic farming compared with conventional or integrated management. It is known that some essential aspects of soil fertility management in organic crop production, in particular rotation design based on grass-clover ley favour the development of wireworms (MAFF 1944). Due to the restriction in insecticide use in Organic Agriculture (OA), wireworm control needs to be managed by agronomic practices and the implementation of biological tools interfering with population dynamics (see also Sufyan et al. in this bulletin).

Some hints in literature e.g. Rawlins (1943) and Jonasson & Olsson (1994) reported varietal differences in tuber susceptibility to wireworm damage. Using tolerant varieties is a common tool in OA and may offer a promising tool to reduce wireworm damages in organic potatoes. Furthermore, it is known that the feeding behaviour of wireworms follows a pattern with active and passive phases that are affected by moulting (Burrage 1963, Doane 1981).

In this paper we present results from field trials focussed on assessing the impact of variety choice and harvest date on wireworm damage in organic potatoes.

Material and methods

The experiments were carried out in 2005 on an experimental field of the Agricultural Chamber in Cologne-Auweiler and on 2 private organic farms in the Rhineland on loess soils. Crops were grown in fields known for high wireworm infestation ranging between 8 and 20
wireworms per m² (10 soil samples, 16*16 cm * 25 cm depth). The main experiment was conducted in Auweiler with 6 mid-early potato varieties (Ditta, Edelstein, Granola, Nicola, Princess, Steffi) after precrop spring barley in a randomised block design with 5 harvest dates ranging between the first decade of August (D1 = 80% of the foliage of a variety destroyed) and the last decade of September (D5) and 4 replications.

Further tuber samples were taken at 3 different dates with 4 replications from 2 private organic farms in Düren (cvs. Granola and Princess) and Wachtberg (cvs. Granola and Nicola) both cultivated after precrop grass-clover ley.

At each sampling date 10 potato plants per plot (2 rows with 5 crops) were harvested, washed, weighted and counted. Subsequent quality assessments of 100 tubers included the number of wireworm and dry core wholes per tuber. Tuber starch content was determined by the underwater weight according to Lunden (1956). Data were analysed by ANOVA followed by Tukey’s-test.

Results and discussion

**Auweiler**

**Tuber yield:** In the context of the research objective of this work the observed significant varietal differences in tuber yield do not deserve further attention. In contrats to these findings it is noteworthy to mention that the harvest date had no effect on tuber yield in any trial, suggesting that crop growth was terminated in the beginning of August (data not shown).

**Tuber quality:** Significant varietal differences in tuber damage caused by wireworms were noted. The variety Princess had higher tuber losses due to wireworm attack (27.0 %) compared to cv. Nicola (12.7%). These findings are of particular interest, because cv. Princess was harvested earlier than cv. Nicola, thereby excluding any effect of harvesting time on tuber damage and suggesting other mechanisms involved.

Figure 1. Influence of variety and harvest date (D1 = early, D5 = late) on tuber losses (mass %) caused by wireworm damage, Auweiler site 2005, columns with different letters are significantly different, Tukey’s test (α < 0.05).
**Düren**

**Tuber yield:** Average tuber yields were comparatively high (34.1 t ha\(^{-1}\)). No influence of either variety or harvest date on crop yield was observed (Table 1).

**Tuber quality:** In accordance with the high wireworm population recorded before planting (20 wireworms per m\(^2\)), average wireworm injuries were higher compared with the other two sites. The highest mass losses due to wireworm damage were recorded in cv. *Princess* (36.7 %) while losses in cv. *Nicola* (22.9 %) were lower (not significant).

Table 1. Influence of variety and harvest date (D1 = early, D3 = late) on potato tuber yield and quality, Düren site 2005, values followed by different letters are significantly different, Tukey’s test (\(\alpha<0.05\)).

<table>
<thead>
<tr>
<th></th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>Princess</th>
<th>Granola</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuber yield (t ha(^{-1}))</td>
<td>35.5</td>
<td>34.9</td>
<td>31.9</td>
<td>36.0</td>
<td>32.2</td>
<td>34.1</td>
</tr>
<tr>
<td>Tuber starch (%)</td>
<td>11.0a</td>
<td>11.0a</td>
<td>10.4b</td>
<td>9.7b</td>
<td>11.9a</td>
<td>10.8</td>
</tr>
<tr>
<td>Losses wireworm (mass %)</td>
<td>22.7b</td>
<td>39.7a</td>
<td>26.9ab</td>
<td>36.7</td>
<td>22.9</td>
<td>29.8</td>
</tr>
<tr>
<td>Losses dry core (mass %)</td>
<td>12.7</td>
<td>11.6</td>
<td>18.5</td>
<td>16.3</td>
<td>12.3</td>
<td>14.3</td>
</tr>
</tbody>
</table>

A significant increase of tuber injuries by wireworms was noted from D1 (mid August) to D2 (beginning of September). However, at D3, tuber losses tended to be lower compared to D2. On average 14.3% of the tubers were damaged by dry core symptoms caused by *Rhizoctonia solani*. Tuber losses by dry core symptoms were neither affected by harvest date nor by variety. The starch content of cv. *Granola* was significantly higher (11.9 %) compared with cv. *Princess* (9.7 %).

**Wachtberg**

**Tuber yield:** Average tuber yield at Wachtberg was low (20.4 t ha\(^{-1}\)). None of the experimental factors variety and harvest date had a significant impact on tuber yield (Table 2).

Table 2. Influence of variety and harvest date (D1 = early, D3 = late) on potato tuber yield and quality, Wachtberg site 2005, values followed by different letters are significantly different, Tukey’s test (\(\alpha<0.05\)).

<table>
<thead>
<tr>
<th></th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>Nicola</th>
<th>Granola</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuber yield (t ha(^{-1}))</td>
<td>21.2</td>
<td>19.3</td>
<td>20.7</td>
<td>19.1</td>
<td>21.8</td>
<td>20.5</td>
</tr>
<tr>
<td>Tuber starch (%)</td>
<td>13.7</td>
<td>13.5</td>
<td>13.5</td>
<td>14.6a</td>
<td>12.6b</td>
<td>13.6</td>
</tr>
<tr>
<td>Losses wireworm (mass %)</td>
<td>10.6</td>
<td>12.8</td>
<td>13.7</td>
<td>8.6b</td>
<td>16.1a</td>
<td>12.4</td>
</tr>
<tr>
<td>Losses dry core (mass %)</td>
<td>4.0</td>
<td>6.4</td>
<td>6.3</td>
<td>4.8</td>
<td>6.3</td>
<td>5.2</td>
</tr>
</tbody>
</table>

**Tuber quality:** Compared with the other two sites tuber losses caused by wireworms were relatively low. Tuber mass losses due to wireworm attack were significantly lower for cv. *Nicola* (8.6 %) compared with cv. *Granola* (16.1 %), while tuber starch content was significantly higher in cv. *Nicola* compared with cv. *Granola*. Tuber losses caused by dry core symptoms were low and were not affected by the experimental factors.
Our experiments have shown that simple agronomic measures such as variety choice and harvest date may help to reduce losses by wireworm damage. However, marketability, i.e. tuber quality and cooking behaviour, as well as resistance to late blight will still remain the key criteria for variety choice in organic potato production. Causes for varietal differences in tuber susceptibility to wireworm damage, e.g. tuber glycoalkaloid content, still deserve further inquiries. A significant correlation between tuber starch content and wireworm damage was not noted.

Under organic growing conditions with limited nutrient supply and pesticide availability tuber growth in the Rhineland area is often finished in the last decade of July either by nitrogen deficiency or by late blight induced leaf destruction. In accordance with these circumstances no yield losses were observed for early compared to late harvest dates in our trials. Under these conditions early tuber lifting date can be an interesting tool to reduce wireworm damage. Sufficiently suberised potatoes with a firm skin and the availability of cooling facilities are indispensable prerequisites for this strategy.

Acknowledgements

This work was supported by the German Federal Ministry of Food, Agriculture and Consumer Protection under the Federal Organic Farming Scheme.

References


Promise versus performance: working toward the use of Metarhizium anisopliae as a biological control for wireworms

Todd Kabaluk¹, Mark Goettel², Jerry Ericsson¹, Martin Erlandson³, Ffion Cassidy¹, Bob Vernon¹, Stefan Jaronski⁴, Kenna Mackenzie⁵, Lee Cosgrove⁶

Agriculture and Agri-Food Canada: ¹Pacific Agri-Food Research Centre, Box 1000, Agassiz, British Columbia, Canada V0M 1A0; ²Lethbridge Research Centre, Alberta; ³Saskatoon Research Centre, Saskatchewan; ⁴United States Department of Agriculture, Sidney, Montana; ⁵Atlantic Food and Horticulture Research Centre, Kentville, Nova Scotia; ⁶address unknown

Abstract: A local isolate of Metarhizium anisopliae has shown promise as a biological control for wireworms based on repeated success in laboratory and greenhouse studies. Several years of field data have shown mixed results when M. anisopliae has been used by a variety of application methods. Laboratory experiments designed to explain these mixed results have pointed to certain biotic and abiotic factors as influential factors. In an attempt to improve efficacy, M. anisopliae strain F52 (also know as BIO1020, BIPESCO-5) was field-tested in 2006 in combination with the insecticides clothianidin and spinosad in potato and corn, and improved yield was found in corn. M. anisopliae isolate characteristics, including growth and sporulation, productivity using solid state fermentation, compatibility with agri-chemicals and pathogenicity toward several species of the European and North American wireworms are presented.

Key words: biological control, microbial insecticide, wireworm, entomopathogen, Metarhizium anisopliae

Introduction

Metarhizium anisopliae is being studied for use as a biological control for wireworms in British Columbia (BC), Canada. There have been reports of this fungus occurring naturally in field populations of wireworms (e.g. Fox and Jaques 1958) and others have made attempts to explore its utility as a biocontrol under laboratory conditions (Zacharuk and Tinline 1960, 1968). More recently, Filipchuk et al. (1995) reported results using M. anisopliae for controlling the tobacco wireworm under field conditions in Russia, but no other reports on field applications have been found.

We have undertaken basic studies to cover the breadth of issues in using M. anisopliae as a wireworm biocontrol (see Kabaluk et al. 2005). While field efficacy of M. anisopliae has been unreliable to date, Ericsson et al. (2007) showed that it’s synergism with spinosad might offer promise. In this context, and with the possibility of using M. anisopliae in a more traditional agricultural setting, we tested it’s compatibility with a variety of agri-chemicals and field-tested fungus-chemical combinations (spinosad and clothianidin) in potato and corn. In this paper, we further report the effect of moisture of different soil types on causing wireworm mycosis, the effect of different isolates on different species of wireworms, and the relative production characteristics of isolates of interest.
Materials and methods

Soil
Soil used in all laboratory experiments, except where otherwise stated, was field-collected silt-loam. Prior to use, it was autoclaved to ensure sterility, followed by screening through fine mesh. Moisture content was adjusted to 13% ± 2% using tap water.

Wireworms
Wireworms were collected from farm fields with no record of pesticide applications. The stock collections were housed in plastic tubs filled with field soil and maintained at 5°C. All experiments used A. obscurus except in the M. anisopliae isolate x wireworm species bioassay which also included A. lineatus, A. sputator, and Limonius canus.

Field trials
i) 2000-2004 M. anisopliae isolate LRC112 conidia were tested for inducing field mortality of wireworms (A. obscurus), and for reducing wireworm feeding damage to new tubers in potato fields. Application methods included conidia granules applied broadcast pre-plant incorporated, in-row, with seed tuber, with wheat rows as an ‘attract and kill’ approach prior to planting potatoes, and in-furrow spray. Rates tested in the field were approximately 10^{14} conidia/ha.

ii) 2007 Corn seed was treated with both agri-chemicals (clothianidin at 2.5 x 10^{-4}g/seed (32.86g/ha), spinosad at 1.522 x 10^{-4}g/seed (20.00g/ha), no agri-chemical) and M. anisopliae isolate F52 (also know as BIO1020, BIPESCO-5) conidia (conidia at 3.805 x 10^8 conidia/seed (5 x 10^{13} conidia/ha), no conidia), constituting a 3 x 2 factorial experiment. The target wireworm species was A. obscurus. The six treatment combinations were arranged in a randomized complete block design and planted at three locations. In a separate experiment, the same treatments (equivalent per hectare rates) were applied to potato seed tubers with the same experimental design and replication. After harvesting corn, the percent stand, plant fresh weight, and per hectare yield were statistically analyzed. After harvesting potato, the number of wireworm feeding holes/tuber, weight/tuber, and plot yield were analyzed.

The effect of selected agri-chemicals on M. anisopliae colony growth and conidia viability
Based on the findings of synergistic interaction between M. anisopliae and spinosad by Ericsson et al. (2007) and the prospective use of M. anisopliae in conventional crop protection systems, we tested agri-chemicals for compatibility with M. anisopliae using F52. Base rates for each chemical were standardized in grams active ingredient/ha, after first obtaining the average of all available label rates of each chemical. The base rate was tested along with higher rates ranging from x5 to x20, depending on chemical availability. Chemicals were dissolved in acetone and mixed with PDA and poured into Petri plates. A droplet of M. anisopliae conidia suspension was placed in the middle of treatment plates and colony growth measured over time with image analysis software. Conidia viability from resulting cultures was assessed after plating suspensions on PDA for 18 hours at 25°C. Effects were determined through single degree of freedom contrasts with the acetone control.

The effect of moisture in three soil types on wireworm mycosis
Three soil types (clay, sandy, organic) were collected from farm fields in the lower mainland of BC, autoclaved, and screened through fine mesh. Moisture was adjusted to either 6%, 12%, or 18% and the soil was then treated with 10^6 M. anisopliae LRC112 conidia/g dry soil. Soil was dispensed into small plastic cups, a single wireworm (A. obscurus) was placed in each cup, and the cup was covered with a fitted lid. Cups were incubated at 20°C and each treatment combination was replicated 15 times with wireworm mycosis being recorded over time. Moisture in the cups was maintained at treatment levels by weighing each cup and adding water to bring the weight back to the original level.
**Persistence of M. anisopliae in field soil**

Fifty 1.5L cores of silt-loam soil were extracted from the field, pooled, and mixed with conidia of *M. anisopliae* isolate LRC112 to achieve a concentration of $2.3 \times 10^6$ conidia/g dry soil. The treated soil was then replaced into the field core cavities. Every three weeks up until the fifteenth week, 10g of soil were sampled from randomly selected cores and the number of *M. anisopliae* colony forming units (cfu’s) determined with dilution plating on Veen’s medium (Veen and Ferron 1966). A final sample was acquired at week 39. The cfu’s in treated soil were adjusted according to ambient levels of *M. anisopliae* in untreated soil.

**M. anisopliae isolate x wireworm species bioassays**

Four isolates of *M. anisopliae* were bioassayed against *A. obscurus*, *A. lineatus*, *A. sputator*, and *L. canus*. The isolates were acquired from cadavers of *A. obscurus* (i-Aobs), *A. lineatus* (i-Alin), *L. canus* (i-Lcan), and F52 (i-F52), and conidia produced for the experiment on PDA cultures. Wireworms were bioassayed in sterile sandy soil with 9% moisture and $10^6$ conidia/g wet soil, with a single wireworm contained in a small plastic cup of treated soil. Fifteen replicate cups were used for each isolate x species combination, and the experiment was conducted twice. Mycosis was recorded over time.

**M. anisopliae isolate production characteristics**

The previously described isolates were compared for productivity through solid state fermentation on sterile barley, colony growth rate on PDA over a range temperatures (thermal gradient profiling), and conidia yield from sporulating cultures.

**Results and discussion**

**Field trials**

i) **2000-2004** Broadcast pre-plant incorporated granules showed a $33.3\% \pm 3.8$ (SE) reduction in the number of wireworm feeding holes/tuber in six independent field trials, but this was not statistically significant because of variability. The same treatment resulted in a significant increase in tuber size in one trial. Other application methods did not appear to confer crop protection but field applications of conidia-coated wheat seed, free conidia, and conidia granules induced wireworm mycosis.

ii) Corn seed treated with *M. anisopliae* isolate F52 conidia resulted in a significant increase in stand density (77.9% - *M. anisopliae*-treated vs. 66.7% - no *M. anisopliae*) and yield (9.6 t/ha - *M. anisopliae*-treated vs. 7.6 t/ha - no *M. anisopliae*), and significantly increased plant fresh weight in spinosad-treated and corn seed with no agri-chemical at one location. Spinosad had no effect on corn yield, whereas clothianidin caused a significant increase in plant stand density and yield. A slight additive effect was observed for either agri-chemical in combination with *M. anisopliae*. In potatoes, this strain of *M. anisopliae* did not affect yield or appear to confer crop protection.

**The effect of selected agri-chemicals on M. anisopliae colony growth and conidia viability**

Table 1 shows that of the insecticides tested, hexahydroxyl, a natural-based neurotransmitter blocker, significantly reduced *M. anisopliae* colony growth. Conidia arising from colonies grown on agar containing thiamethoxam showed a 10% reduction in germination. Agar containing the fungicides captan and thiophanate methyl severely restricted *M. anisopliae* colony growth.
Table 1. The effect of selected agri-chemicals on colony growth of *Metarhizium anisopliae* and germination of conidia arising from those colonies.

<table>
<thead>
<tr>
<th>Agri-chemical</th>
<th>Rates tested</th>
<th>Effect on area of <em>M. anisopliae</em> colony growth*</th>
<th>Effect on germination of conidia growing from colonies*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insecticides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clothianidin</td>
<td>x1, x5, x10, x15, x20</td>
<td>Possible reduction at x1 rate</td>
<td>No effect</td>
</tr>
<tr>
<td>Halofenozide</td>
<td>x1, x10</td>
<td>No change</td>
<td>No effect</td>
</tr>
<tr>
<td>Hexahydroxyxyl</td>
<td>x1, x10</td>
<td>Clear reduction at x10 rate</td>
<td>No effect</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>x1, x5, x10, x15, x20</td>
<td>Slight reduction at x1 rate</td>
<td>No effect</td>
</tr>
<tr>
<td>Spinosad</td>
<td>x1, x5, x10, x15, x20</td>
<td>No change</td>
<td>No effect</td>
</tr>
<tr>
<td>Thiamethoxam</td>
<td>x1, x10</td>
<td>No change</td>
<td>10% reduction</td>
</tr>
<tr>
<td>Fungicides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Captan</td>
<td>x1, x10</td>
<td>Almost no growth</td>
<td>No conidia produced</td>
</tr>
<tr>
<td>Thiophanate methyl</td>
<td>x1, x10</td>
<td>Almost no growth</td>
<td>No conidia produced</td>
</tr>
</tbody>
</table>

*The effect of moisture in three soil types on wireworm mycosis*

In sand, all three moisture contents enabled wireworm mycosis. In clay and organic soils, wireworm mycosis was enabled at 12% and 18% only (Figure 1). At all three moisture contents, the sand felt moist, with free water in the 18% moisture treatment. In clay and organic soils however, 6% moisture constituted very dry soil. We concluded that infection and mortality is able to proceed with medium to very high soil moisture content, but is inhibited in dry soil.

*Persistence of *M. anisopliae* in field soil*

Overall, *M. anisopliae* persisted well in field soil (Figure 2). Within the first three weeks, there was a decline from the initial concentration but levels rose over time returning to near-initial levels after 15 weeks. The last sampling date showed a moderate (about 20%) decrease from the initial concentration.

*M. anisopliae isolate x wireworm species bioassays*

Isolates derived from cadavers of *A. obscurus*, *A. lineatus*, and *L. canus* (i-Aobs, i-Alin, and i-Lcan) caused moderate levels of mycosis in *A. lineatus* and *A. sputator*, and high levels in *A. obscurus*. All isolates caused lower levels of mycosis in *L. canus*. i-F52 caused low levels of mycosis in all wireworm species (Figure 3). i-Aobs and i-Alin, while acquired from cadavers of different species, have been showed to be genetically similar according to AFLP analysis (Inglis et al., unpublished). These two isolates were acquired from cadavers from the lower mainland of BC, while i-Lcan was acquired from the interior BC. i-F52 is a commercial isolate.
Figure 1. The effect of moisture content in three soil types on wireworm mycosis.

Figure 2. The persistence of *Metarhizium anisopliae* applied as conidia to field soil.
Figure 3. The effect of four isolates of *Metarhizium anisopliae* on four species of wireworms (i-Aobs acquired from cadaver of *Agriotes obscurus*; i-Alin acquired from cadaver of *A. lineatus*; i-Lcan acquired from cadaver of *Limonius canus*; i-F52 is a commercial isolate).

**M. anisopliae isolate production characteristics**

F52 was more productive in terms of colony growth rate, conidia yield per unit colony area, and conidia production on barley substrate (Figure 4). Isolates i-Aobs, i-Alin, and i-Lcan, while showing similar maximum colony growth rate to i-F52, were inferior in terms of conidia production. All isolates exhibited maximum colony growth rate at 25°C.

**Discussion**

While insecticidal activity of *M. anisopliae* toward wireworms has shown good promise in laboratory bioassays and simulated field experiments in the greenhouse, field efficacy has fallen short despite testing many application methods. Treating corn seeds with conidia increased the yield of corn, and if that effect is the result of causing wireworm mortality (in contrast to a repellent or nutrient effect), then an even greater effect can be expected by using the more virulent isolates (i-Aobs, i-Alin, or i-Lcan). While more virulent, these isolates are inferior to F52 in terms of productivity, and this fact is problematic in considering the more virulent isolates in a commercial context. Furthermore, with prospective commercialization, the target wireworm species will have to be considered as they differ in their susceptibility to individual isolates. Despite these shortcomings, optimal use patterns, through the consideration of biotic and abiotic factors (such as soil type and moisture), are being developed to maximize efficacy. There is also promise for combining *M. anisopliae* with sublethal doses of selected agri-chemicals. The fungus shows good persistence following application to field soil.

Elaboration on these findings can be obtained by contacting the senior author at kabalukt@agr.gc.ca
Figure 4. Production characteristics of four isolates of *Metarhizium anisopliae*: under thermal-gradient profiling (top graph), conidia yield from colonies grown on nutrient agar (middle graph), and conidia yield from solid state fermentation on sterile barley (bottom graph). The source of the isolates is described in the Figure 3 caption.

**Acknowledgements**

This research was financially supported by the Improving Farming Systems and Practices grant and Matching Investment Initiative of Agriculture and Agri-Food Canada, and the Organic Farming Research Foundation, California.

**References**


Evaluation of *Metarhizium anisopliae* isolates for biocontrol of *Agriotes* based on genetic, biochemical and virulence characters

Vandana Ghormade¹, Werner Jossi¹, Santosh Chavan², Arumugam Rajendran², Amey Ghondhelekar², Franco Widmer¹, Siegfried Keller¹, Jürg Enkerli¹

¹Molecular Ecology, Agroscope Reckenholz-Tänikon Research Station ART, Reckenholzstrasse 191, CH 8046 Zürich, Switzerland; ²Biochemical Sciences Division, National Chemical Laboratory, Pune 411008, Maharashtra, India.

**Abstract:** The fungus *Metarhizium anisopliae* naturally infects *Agriotes* spp. and it has a great potential for use as a BCA against this pest insect. Fungal strains that are evaluated for biocontrol typically are selected based on their virulence towards the target host, which is assessed with bioassays. However, in the case of *Agriotes*, bioassays are very laborious and time consuming.

Our goal was to assess alternative criteria i.e. biochemical and genetic strain characteristics as well as virulence assessment on the alternative host *Helicoverpa armigera* for pre-selection of *M. anisopliae* strains suitable for *Agriotes* control. A collection of 22 *M. anisopliae* isolates was used to investigate and compare the various characteristics. Genetic characterization was based on microsatellite markers as well as on PCR-RFLP analyses of two genes coding for proteases and three genes coding chitinases and biochemical characterization was based on protease and chitinase enzyme activities. The virulence on *H. armigera* was determined for all 22 isolates however, virulence on *Agriotes* spp. was determined only for a subset of 7 isolates due to a limited number of *Agriotes* spp. larvae available. Assessment of correlations between all the different characteristics revealed significant correlations between chitinase activity and virulence on both hosts, indicating that chitinase activity may be used as an alternative criterion for pre-selection of *M. anisopliae* strains for *Agriotes* control. There was no significant correlation between virulence on *H. armigera* and virulence on *Agriotes*. However, bioassays with *H. armigera* allowed for identification and elimination of strains with low virulence. Therefore, bioassay with *H. armigera* might be a suitable tool for the pre-selection of virulent *M. anisopliae* strains. The number of isolates investigated and compared in this study was low and therefore conclusions have to be considered preliminary.

**Key words:** PCR-RFLP, microsatellite, chitinase, protease, bioassay, *Agriotes* spp.

**Introduction**

Wireworms, the larval stages of click beetles (*Agriotes* spp., Coleoptera, Elateridae) are fast emerging pests of crops such as potato and maize in Europe. They live in the soil for 4-5 years where they cause severe damage by feeding on plant roots and tubers. Recently, Keller and Zimmerman (2005) have estimated that in Europe wireworms have colonized 9600 ha of arable land of which 2000 ha have suffered severe economic damage. Increasing environmental awareness has resulted in a more restricted use of pesticides As a consequence the development of alternative control strategies has become an essential task. Biocontrol of wireworms with entomopathogenic fungi is one possible strategy to control wireworm damage. The insect pathogenic fungus *Metarhizium anisopliae* naturally infects *Agriotes* larvae as well as adults and therefore represents a potential biocontrol agent (BCA). Selection and characterization of suitable strains is an essential step towards the development of a successful BCA. Typically strains are selected based on their virulence towards the target
host, which is assessed with bioassays. The use of such assays for assessment of virulence of \textit{M. anisopliae} strains towards \textit{Agriotes} has been reported previously (Kabaluk et al., 2005). However, \textit{Agriotes} bioassays are very laborious and time consuming as single assay take 4-5 months for completion and \textit{Agriotes} spp. are difficult to rear.

Molecular genetic and biochemical techniques are frequently used to characterize fungal strains and to provide tools that allow for identification of a selected strain, which is important for monitoring and quality control during development of a BCA (Bidochka 2001). A variety of genetic tools have been applied for genetic characterization of \textit{M. anisopliae} in the past. They include techniques like analyses of the rRNA gene complex (Mavridou and Typas, 1998), Random Amplification of Polymorphic DNA (RAPD, Bidochka et al., 1994) and microsatellite analyses (Enkerli et al., 2004) as well as Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP) analyses of virulence genes coding for proteases \textit{Pr1A} and \textit{Pr1B} (Leal et al., 1997) and chitinases \textit{Chi18-III}, \textit{Chi18-Va} and \textit{Chi18-Vb} (Ghormade et al., in preparation). Biochemical characterization of \textit{M. anisopliae} has been carried out by measurement of enzyme activities of proteases, lipases, and chitinases, which are known virulence factors involved in cuticle degradation during penetration of the host (St Leger et al., 1986; Nahar et al., 2003).

Our aim is to evaluate and select \textit{M. anisopliae} strains virulent on \textit{Agriotes} that may be used to develop a BCA against this pest. Because virulence assays with \textit{Agriotes} are very laborious and time consuming our first goal was to assess alternative criteria for evaluation and selection of virulent \textit{M. anisopliae} strains. In a first step we have investigated the potential of biochemical and genetic strain characteristics as possible selection criteria for virulent strain identification. In the second step we have assessed the potential of an alternative and readily available host such as \textit{Helicoverpa armigera} (Lepidoptera, Noctuidae) for use in bioassays to pre-select virulent \textit{M. anisopliae} strains.

**Materials and Methods**

**Strain isolation**

A total of 22 \textit{M. anisopliae} isolates were included in this study. 16 \textit{M. anisopliae} strains originated from infected larvae or adults of \textit{Agriotes lineatus}, \textit{Agriotes obscurus} and \textit{Agriotes sputator} collected from different locations in Switzerland. Furthermore, 4 strains originated from \textit{Amphimallon} spp. (Coleoptera, Scarabaeidae), 1 strain from \textit{Phyllopertha horticola} (Coleoptera, Scarabaeidae) and 1 strain isolated from a soil sample from Saswad, Maharashtra, India (Ma2062) were also evaluated.

**Genetic characterization**

PCR-RFLP analysis of genes coding for the protease \textit{Pr1A} and \textit{Pr1B} was performed as described by Leal et al. (1997) and Wang et al. (2002). PCR-RFLP analysis of genes coding for the chitinase \textit{Chi18-III}, \textit{Chi18-Va} and \textit{Chi18-Vb} was carried out according to protocols recently developed (Ghormade et al., in preparation). Each locus was analyzed by use of 3 restriction enzymes. Resulting banding patterns were recorded as binary matrices based on presence or absence of corresponding fragments. Microsatellite analyses were performed at 13 loci as described by Enkerli et al. (2004) and data was recorded as binary matrix based on presence or absence of corresponding allelic products.

**Biochemical analysis**

For each isolate 100 ml chitin containing medium was inoculated with \(10^7\) \textit{M. anisopliae} conidia / ml and cultures were grown for 96h at 28°C (Nahar et al., 2003). Chitinase activity was measured using colloidal chitin as a substrate and p-dimethyl amino benzaldehyde (DMAB) for colorimetric determination of released \(\text{N-acetylglucosamine residues (GlcNAc)}\)
(Vyas and Deshpande, 1989). Protease activity was measured by using casein as a substrate. After addition of trichloroacetic acid (TCA) to a concentration of 2.5%, optical density was recorded as absorbance of the TCA soluble fraction at 280nm.

**Bioassays**

Virulence of *M. anisopliae* isolates was determined in bioassays with *H. armigera* and *Agriotes* spp. by assessing larval mortality for each strain. Thirty third instar or sixth instar larvae of *H. armigera* and *Agriotes* spp. respectively, were dipped for 5 seconds in a conidial suspension (10^7 conidia / ml). Excess water was removed with a paper towel and larvae were placed individually in empty vials (*H. armigera*) or vials containing sterile soil (*Agriotes* spp.) and kept in dark at 25 °C. *H. armigera* larvae were fed with disinfected okra pieces and mortality was recorded after 14 days. *Agriotes* spp. larvae were incubated in the dark at 22 °C without feed and mortality was recorded after 16 weeks.

**Statistical analysis**

Explorative statistical analysis of RFLP data was performed with Ward cluster analysis based on squared euclidean distances with Statistica version 6.1 (StatSoft Inc, Tulsa, OK). Similarities among distance matrices were determined with the NTSYS-pc 2.2 software using the Mantel test statistics. Pearson calculation in Statistica version 6.1 was used for correlation of biochemical and virulence data.

**Results and discussion**

The 22 *M. anisopliae* strains were genetically characterized by performing PCR-RFLP analyses on two protease (*Pr1A*, *Pr1B*) and three chitinase coding genes (*Chi18-III*, *Chi18-Va* and *Chi18-Vb*) as well as microsatellite analyses (Table 1). PCR-RFLP analyses of protease-coding genes allowed for discrimination of 6 genotypes (P1-6) among the 22 isolates while analyses of chitinase-coding genes allowed for discrimination of 7 genotypes (C1-7). Application of 13 microsatellite markers resulted in discrimination of 6 genotypes (M1-6). PCR-RFLP data based distance matrices of protease and chitinase coding genes correlated with high significance (Mantel test r = 0.95***). Similarly, PCR-RFLP analyses derived distance matrices of protease and chitinase-coding genes significantly correlated with those obtained from microsatellite analyses (Mantel test r = 0.85***, r = 0.91*** respectively). The combination of PCR-RFLP data for chitinase-coding genes with microsatellite data allowed to identify 8 genotypes among the 22 *M. anisopliae* strains. The combined data set discriminated 4 different genotypes (I, VI, VII, VIII) among the 16 *M. anisopliae* strains isolated from *Agriotes* spp. and 5 genotypes among the 6 strains isolated from other hosts or from soil (II, III, IV, V, VIII; Table 1). PCR-RFLP data of protease-coding genes did not provide additional discrimination.

Chitinase activity among the 22 *M. anisopliae* isolates ranged from 0.22 to 5.26x10^{-3} U/ml while protease activities ranged from 0.39 to 3.38 U/ml. Protease and chitinase activities showed a weak but significant correlation (Pearson r = 0.5*). Enzyme activities data did not correlate with the genetic data.

Virulence of the 22 *M. anisopliae* strains tested on *H. armigera* ranged from 36 to 95% mortality and correlated significantly with chitinase activity (r = 0.6**) but it did not with protease activity. However, no significant correlation was detected between virulence and genetic data. Based on virulence in the *H. armigera* bioassay 7 strains were selected for a bioassay with *Agriotes* spp.. These included strains with high (> 80% mortality, Ma 5016 and 997), medium (60 – 79% mortality, Ma 5015, 5021 and 5026) and low mortality (< 60% mortality, Ma 5017 and 714). Six of the 7 selected strains originated from *Agriotes* spp. and
all displayed genotype I. Strain Ma5026 isolated from *Amphimallon* sp. displayed genotype VIII.

Table 1. Genotypes for 22 *M. anisopliae* strains based on PCR-RFLP analyses of two protease and three chitinase-coding genes as well as microsatellite analyses.

<table>
<thead>
<tr>
<th>No. of Isolates&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Proteases genotype &lt;sup&gt;(Pr1A &amp; Pr1B)&lt;/sup&gt;</th>
<th>Chitinases genotype &lt;sup&gt;(ChiIII, ChiVa &amp; ChiVb)&lt;/sup&gt;</th>
<th>SSR genotype (13 SSR markers)</th>
<th>Combined genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>P1</td>
<td>C1</td>
<td>M1</td>
<td>I</td>
</tr>
<tr>
<td>2 (2 A)</td>
<td>P1</td>
<td>C1</td>
<td>M2</td>
<td>II</td>
</tr>
<tr>
<td>1 (1 P)</td>
<td>P1</td>
<td>C2</td>
<td>M2</td>
<td>III</td>
</tr>
<tr>
<td>1 (1 S)</td>
<td>P2</td>
<td>C3</td>
<td>M3</td>
<td>IV</td>
</tr>
<tr>
<td>1 (1 A)</td>
<td>P3</td>
<td>C4</td>
<td>M4</td>
<td>V</td>
</tr>
<tr>
<td>1</td>
<td>P4</td>
<td>C5</td>
<td>M5</td>
<td>VI</td>
</tr>
<tr>
<td>1</td>
<td>P5</td>
<td>C6</td>
<td>M5</td>
<td>VII</td>
</tr>
<tr>
<td>4 (1 A)</td>
<td>P6</td>
<td>C7</td>
<td>M6</td>
<td>VIII</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of isolates originating from other hosts are specified in parenthesis; *Amphimallon* spp. (A), *Phyllopertha horticola* (P) or soil sample India (S)

The 7 selected isolates could be divided into two groups based on the bioassays with *Agriotes* spp., i.e. a group with high virulence (≥ 65% mortality) and a group with low virulence (≤ 45% mortality). Of the three isolates displaying high virulence on *Agriotes* spp. isolate Ma5016 showed high virulence and isolates Ma5015 and Ma5021 showed medium virulence on *H. armigera*. The virulence of the 4 isolates with low virulence on *Agriotes* spp. ranged from low (Ma5017, Ma714) to medium (Ma5026) to high (Ma997) on *H. armigera*. Chitinase activity significantly correlated with virulence on *Agriotes* spp. (Pearson r = 0.55**) whereas protease activity did not. No significant correlation was observed between data obtained from *H. armigera* bioassay and *Agriotes* spp. bioassay.

Figure 2. Biochemical activities of selected *M. anisopliae* strains and their virulence on *H. armigera* and *Agriotes* spp.. □ Chitinase activity (10⁻³ U/ml), □ Protease activity (U/ml), □ % Mortality *H. armigera*, □ % Mortality *Agriotes* spp.
Our goal was to assess alternative criteria for the evaluation and selection of *M. anisopliae* isolates for *Agriotes* biocontrol, which may be used to pre-select isolates and thus reduce the time consuming screening with *Agriotes*. For that purpose various genetic and biochemical characteristics were assessed and statistically analyzed for possible correlations with virulence. A significant correlation was detected between chitinase activity and virulence on *H. armigera* and *Agriotes* spp.. However, no correlation was found between virulence on the two hosts and protease activity or any of the genetic analyses. Importance of chitinase and protease activities for high virulence is well documented (Nahar et al., 2003; St Leger et al., 1986) and it is interesting to notice that in this study a correlation was only found for chitinase activity. However, protease activity was detected in all the isolates and possibly a basal level of protease activity in combination with high chitinase activity is sufficient to provide high virulence on the tested hosts. Results of this study indicate that chitinase activity may be an alternative criterion for pre-selection of *M. anisopliae* strains for *Agriotes* control.

Due to the limited number of *Agriotes* spp. larvae available only seven of the 22 isolates were selected to assess correlation between virulence on *Agriotes* spp. and *H. armigera*. The virulence assays with the 2 hosts did not correlate, however the assay with *H. armigera* allowed identification and elimination of strains with low virulence. Therefore, bioassay with *H. armigera* may be a suitable tool for the pre-selection of virulent *M. anisopliae* strains. The number of isolates investigated in this study was low and therefore conclusions have to be considered preliminary. Assays and comparisons will have to be repeated with more isolates to allow for final conclusions. Furthermore, other hosts like *Tenebrio molitor* or *Galleria mellonella* might be assessed to provide additional flexibility in the choice of alternative hosts for pre-screening *M. anisopliae*.

**Acknowledgements**

We thank Dr. M.V. Deshpande, National Chemical Laboratory, Pune 411008, India for providing the opportunity to carry out biochemical analyses and *H. armigera* bioassay in his laboratory. This project is a part of the Indo-Swiss Collaboration in Biotechnology and the funding was received from the Swiss Agency for Development and Cooperation (SDC), Berne, Switzerland. SC, RA and AG thank the Department of Biotechnology, New Delhi, India and SDC, Switzerland for the financial support.

**References**


Investigations on click beetles using pheromone traps

Muhammad Sufyan¹, Daniel Neuhoff¹, Lorenzo Furlan²
¹ Institute of Organic Agriculture, University of Bonn, Katzenburgweg 3, D-53115 Bonn, Germany; ² Dep. of Agronomy, Entomology, University of Padua, Agripolis, via Romea 16, Legnaro PD, Italy.

Abstract: Wireworms may cause damages in organic crops such as potatoes, carrots and maize. Since no synthetic pesticides are allowed in Organic Agriculture, control strategies need to be targeted on agronomic approaches and on biological tools interfering with the population dynamics. In North Rhine - Westphalia / Germany several strategies were investigated at the Institute of Organic Agriculture / University of Bonn, including an evaluation of mass trapping/sex disruption by using pheromone traps for Agriotes lineatus, A. obscurus and an assessment of the range of attractiveness of the pheromone traps used. Experiments on seasonal fluctuation of adult population between 2004 and 2006 indicated that the swarming period lasted from late April to end of August with one main peak in May and one weaker peak in mid June respectively. A tendency to decreased wireworm population was noted in plots with the permanent presence of sex pheromone traps and the continual removal of the males over three years compared with an untreated control area (no male trapping). Experiments focussed on estimating the range of attractiveness of the pheromone traps indicated that the recovery rate of the released beetles (A. lineatus and A. obscurus) to the traps was rather dependent on release distance than on time. Recovery rates for both species > 60% were only noted in clover grass for release distances up to 10m, while less than 10% of the beetles released at a distance of 60 m returned to the trap. Recovery rates of beetles released on bare soil were generally lower compared with clover grass. More than 50% of the males were recovered within the first 24 hours.

Key words: Agriotes spp., sex pheromone traps, wireworm control

Introduction

Wireworms, the larvae of click beetles (Coleoptera, Elateridae), are important dwellers of arable soils (Parker & Howard 2001), feeding on potato tubers, maize roots and other arable crops. They often cause economically severe damage (Hill 1987), especially in Organic Farming, where no synthetic insecticides are allowed for direct control. There are different species of Agriotes damaging plants in Europe but A. lineatus, A. obscurus and A. sputator are prevailing in Germany. In organic farming systems pest control strategies are mainly based on preventative rather than on curative methods (Sharma 2001).

Effective, easy and inexpensive tools are needed to identify those fields that have high wireworm populations in order to ensure that treatments are only applied where necessary or to avoid the cultivation of susceptible crops in heavily infested fields. Sex pheromone traps represent a potential solution to these problems as they monitor the only stage that lives outside the soil, i.e. the adults (Furlan et al., 1996; Furlan et al. 1997). Pheromone trapping system proved to be effective and reliable for most of the Agriotes species and clearly offered a method of assessing click beetle populations without the difficulties associated with soil sampling for wireworms (Furlan et al., 1997; Furlan & Toth 1999).
The objectives of the work were i) to improve the effectiveness of pheromone traps for click beetle monitoring, and ii) to evaluate the potential of pheromone traps for interfering with *Agriotes* populations.

**Material and methods**

**Experimental site**
The trials were carried out at the experimental farm 'Wiesengut' / Hennef (7° 17’ East, 50° 48’ North), belonging to the University of Bonn, Germany. The farm is under organic management since 1986. The soils consist of acid alluvial loams of varying depths with heterogeneous gravel layers at the soil surface (FAO: Fluvisol), pH = 6.2. The crop rotation includes winter rye, grass-clover ley, potatoes, winter wheat, faba beans and spring wheat. At this site two different experiments were carried out.

**Prevention trial**
The experiment is conducted since 2004 on a grass-clover ley (app. 1.5 ha), which includes 4 plots each one with an area of 20 x 30 = 600 m². In 2 two plots YATLOR funnel traps (one per plot) for *A. lineatus*, *A. obscurus* and *A. sputator* (Furlan et al. 2001) were placed out. Male click beetles were captured and removed from the traps weekly. Two untreated plots with no pheromone traps were located at a distance of 80 m. Soil samples (20 cm depth) were taken in August 2004 (50 per plot) as well as June and September 2006 (40 per plot) and assessed for wireworms.

**Estimation of range of attractiveness**
This trial was conducted on bare soil and on a grass-clover ley during May-June 2006 with the species *A. lineatus* and *A. obscurus*. Homogeneous groups of male beetles (25 per distance) were painted in different pattern and released upwind and downwind at different distances (2m, 5m, 10m, 15m, 20m and 60m) from the pheromone traps. The recovery rate to the traps was regularly assessed over 5 weeks.

**Results and discussion**

**Prevention Trial**

**Male Trapping:** The highest total number of beetles captured per season in both "treated" plots with traps was recorded in 2004 with 2373 individuals (Table 1). The highest amount of caught males was noted in the beginning of May 2005 (812 individuals). The swarming period lasted from late April to end of August with one main peak in May and one weaker peak in mid June respectively in all three years and no difference in swarming dynamics was observed among species (Figure 1). Pheromone traps proved to be highly selective and very few sporadic catches were observed.

**Wireworms:** Three months after the beginning of the experiments the total number of wireworms found was 0.58 per soil core in the treated and 0.31 in the untreated plots respectively (Table 2). Two years later (May 2006) the total number of wireworms detected tended to be higher in untreated plots than in treated plots. The number of wireworms of the trapped beetle species *A. lineatus* and *A. obscurus* was significantly lower in the treated than in the untreated plots.

Comparable findings were noted at the second assessment date in September 2006, but given the two replications only, the differences for *A. lineatus* and *A. obscurus* between cleaned plots (0.13 wireworms per soil core) and control plots (0.28 per soil core) were not significant. The assessments will be continued in 2007.
Table 1. Total number of click beetles captured weekly in pheromone traps over the season.

<table>
<thead>
<tr>
<th>Years</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>T7</th>
<th>T8</th>
<th>T9</th>
<th>T10</th>
<th>T11</th>
<th>T12</th>
<th>T13</th>
<th>T14</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>78</td>
<td>365</td>
<td>256</td>
<td>262</td>
<td>36</td>
<td>110</td>
<td>431</td>
<td>102</td>
<td>163</td>
<td>291</td>
<td>212</td>
<td>56</td>
<td>10</td>
<td>2372</td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>812</td>
<td>117</td>
<td>108</td>
<td>51</td>
<td>251</td>
<td>157</td>
<td>134</td>
<td>44</td>
<td>66</td>
<td>3</td>
<td>1743</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2006*</td>
<td>45</td>
<td>805</td>
<td>379</td>
<td>29</td>
<td>23</td>
<td>97</td>
<td>268</td>
<td>88</td>
<td>101</td>
<td>105</td>
<td>76</td>
<td>42</td>
<td>54</td>
<td>16</td>
<td>2128</td>
</tr>
</tbody>
</table>

* two sampling dates merged to one
2004: T1 = 14.05.04 …T13 = 24.08.04;
2005: T1 = 04.05.05…T10 = 30.08.05;
2006: T1 = 02.05.06…T14 = 07.08.06

Figure 1. Click beetles (A. lineatus and A. obscurus) captured per day on a grass-clover ley in pheromone traps in 2006.

Table 2. Number of wireworms detected per soil core, prevention trial 2004 - 2006, Wiesengut / Hennef.

<table>
<thead>
<tr>
<th>Plot</th>
<th>August 2004</th>
<th>June 2006</th>
<th>September 2006</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All wireworm species</td>
<td>All wireworm species</td>
<td>All wireworm species</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>untreated</td>
<td>A. lineatus + A. obscurus</td>
</tr>
<tr>
<td>1</td>
<td>0.76</td>
<td>0.28</td>
<td>0.18</td>
</tr>
<tr>
<td>2</td>
<td>0.40</td>
<td>0.34</td>
<td>0.30</td>
</tr>
<tr>
<td>Mean</td>
<td>0.58</td>
<td>0.31</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Values followed by different letters are significantly different, Tukey’s test (α<0.05).
Estimation of range of attractiveness
The range of attractiveness of the pheromone traps for *A. lineatus* and *A. obscurus* was assessed in 2006 (Figures 2 to 3). Recovery rates for both species > 60% were only noted in clover grass for release distances up to 10 m, while less than 10% of the beetles released at 60 m returned to the trap. Recovery rates of beetles released on bare soil were generally lower. The results also showed that > 50% of the beetles were recovered on the first day and that at low distances (up to 10 m) recovery rates were significantly higher.

![Figure 2](image2.png)

Figure 2. Influence of the release distance on the recovery rate of *A. lineatus*. Values within a line followed by the same letter are not significantly different, Tukey's test ($\alpha < 0.05$).

![Figure 3](image3.png)

Figure 3. Influence of the release distance on the recovery rate of *A. obscurus*. Values within a line followed by the same letter are not significantly different, Tukey's test ($\alpha < 0.05$).
The experiments supply first reliable data on the range of the attractiveness of the sex pheromone traps for some of the most widespread *Agriotes* species. Data show that usually beetles are actively attracted to the sex pheromone traps within a range up to 10 m. Recovery rates from longer distances are low. Although a potential for mass trapping to decrease wireworm population seems to occur, greater experimental efforts are needed to define whether this strategy might be feasible from a practical point of view.

**References**


Melolontha and other Scarabaeidae
The swarming flight of Common cockchafer *Melolontha melolontha* L., 1758 (Coleoptera, Scarabaeidae) in two different areas of Bavaria and an approach to control the egg deposition

Ullrich Benker, Bernhard Leuprecht
Bavarian State Research Centre for Agriculture (LfL), Institute for Plant Protection, Lange Point 10, D-85354 Freising, Germany

Abstract: In Bavaria there are two well known large populations of the common cockchafer *Melolontha melolontha*. One of them is located in the Hessenthal-Mespelbrunn valley in the north-west of Bavaria (Spessart), where in the last four years the Institute for Plant Protection in Freising made several trials to control the grubs. The other population is located south of Augsburg in the rural district of Landsberg/Lech within and around the small village Reichling. In 2006 in both places the swarming flight of *M. melolontha* was expected and this was a good chance to observe the behavior of the beetles in these two completely different landscapes.

In Hessenthal-Mespelbrunn cockchafers represent an important pest in the grassland and therefore, farmers need efficient control measures. The grubs feed on the roots of plants and in addition the sward is destroyed and turned upside down by wild pigs digging for the grubs.

Pilot experiments to control *M. melolontha* were carried out with four insecticides with the active ingredients neem (Neem Azal), \(\lambda\)-cyhalothrin (Karate Zeon), dimethoat (Perfekthion) and *Bacillus thuringiensis* (Novodor) to evaluate the most effective treatment for possible future large scale applications. The most effective insecticide in this experiment was \(\lambda\)-cyhalothrin, followed by dimethoat. However, it is uncertain whether these insecticides will be applied in the future, because there are public concerns and objections from different organisations.

Another approach was tested within the most threatened area in the Hessenthal-Mespelbrunn valley. The aim of this approach was to attract egg laying females to defined plots with short cut grass, in which after egg deposition and hatching of the larvae mechanical control as well as biological control measures in the form of *B. brongniartii* could be applied in the following spring.

In the Lechgau area at Reichling the cockchafer *M. melolontha* is not considered an agricultural pest, although in 2006 the trees in Reichling were more seriously infested with beetles compared to the Spessart region. The more or less flat grassland in Reichling receives enough rainfall, the meadows are dominated by dandelion and the damage of the grubs in the grassland is negligible. Farmers in this area tolerate the grubs. The damage caused by the beetles nearly disappears with a second shoot of the foliage and garden owners also see no need for an extensive control of the cockchafers. In fact, quite the contrary was the case as tourists were coming from distances of about 100 kilometres to collect beetles.

Key words: *Melolontha melolontha*, swarming flight, egg deposition, integrated control

Introduction

The common cockchafer *Melolontha melolontha* L., 1758 (Coleoptera, Scarabaeidae) is the most popular scarab species in Bavaria. Two large populations are well known for decades and the area of their circulation is closely localised. One of these populations, which causes severe damage in grassland, is located in north west Bavaria in the Hessenthal-Mespelbrunn valley in a low mountain range called Spessart. The Hessenthal-Mespelbrunn valley is typical for a Spessart valley with its small town at the bottom and its sloping landscape with meadows, small fields and orchards. Furthermore the valley is completely surrounded of large
beech tree forests. Since 2001 the Institute for Plant Protection has performed several trials to control the grubs in this valley (Benker & Leuprecht, 2004; Benker & Leuprecht, 2005). The use of the entomopathogenic fungus *Beauveria brongniartii* was the main control strategy besides using insecticides or a rotary hoe for mechanical treatment. However, the success with the applied approaches up to now was not overwhelming but still promising.

The second big population of *M. melolontha* is located within and around Reichling, a village near the river Lech in south Bavaria in the rural district of Landsberg/Lech. In the Hessenthal-Mespelbrunn valley as well as in the Reichling area the last swarming flight was observed in 2003 and the recent swarming flight 2006 occurred as expected.

Populations of other species of the Scarab family like the June beetle *Amphimallon solstitiale* (L., 1758), the garden chafer *Phyllopertha horticola* (L., 1758) and the Welsh chafer *Hoplia philanthus* (Fuessly, 1775) occur from time to time in Bavaria, also in the Hessenthal-Mespelbrunn valley. Sometimes they exceed the threshold for economic damage in grassland like sports field or golf courses. The two areas mentioned above are the areas with the most reliable occurrence of Scarabs, particularly *Melolontha* in Bavaria. As the two areas are quite different in landscape and other field conditions it was a good chance to compare the swarming flights 2006 with each other.

**Material and methods**

**Control of the adult beetles**

In the run-up to the swarming flight it was considered to spray insecticides with a helicopter as an emergency plan to drastically reduce the emerging beetle population. However, the insecticide application by helicopter was cancelled because no appropriate compound was registered at that time and the necessary financial support for the treatments was missing. In order to provide knowledge for selection of a suitable insecticide for spraying the adult chafer, the Institute for Plant Protection in cooperation with the Office for Agriculture in Würzburg performed a greenhouse screening experiment. It started just after the beginning of the swarming flight April 28th 2006.

Some twigs with beech leaves were treated with the active ingredients listed in Table 1. and put into a wooden insect cage. In every cage 25 individuals of *M. melolontha*, 9 females and 16 males, were subsequently exposed to the spray on the leaves. After the treatment the cages were transported to a greenhouse in Freising with outside temperature and daylight, protected only against rainfall. Beetle mortality was monitored daily and twigs with dried leaves were replaced with fresh ones when necessary.

Table 1. Ingredients tested for cockchafer control

<table>
<thead>
<tr>
<th>Var.</th>
<th>Active ingredient</th>
<th>Product name</th>
<th>Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dimethoat</td>
<td>Perfekthion</td>
<td>0.6 l/ha</td>
</tr>
<tr>
<td>2</td>
<td>λ-Cyhalothrin</td>
<td>Karate Zeon</td>
<td>0.075 l/ha</td>
</tr>
<tr>
<td>3</td>
<td>Azadirachtin</td>
<td>NeemAzal</td>
<td>1.5 l/ha</td>
</tr>
<tr>
<td>4</td>
<td><em>Bacillus thuringiensis</em></td>
<td>Novodor</td>
<td>5.0 l/ha</td>
</tr>
<tr>
<td>5</td>
<td>Control</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>
**Controlling the egg deposition**

It always has been assumed that female beetles of *M. melolontha* prefer for their egg deposition open greenland sites with short grass and high thermal radiation. Before the swarming flight some farmers in the Hessenthal-Mespelbrunn valley prepared in cooperation with the Office for Agriculture in Karlstadt/Aschaffenburg six suitable grassland plots of about 50 x 20 metres to attract females and to obtain a locally defined and controlled egg deposition. “Suitable” means widely flat land with only a few stones in the soil. “Prepared” means that the plots were mulched and cut in time to have short grass in the plots at the time of the start of the beetle flight. A treatment to control *M. melolontha* in the grassland plots with a rotary hoe or with *B. brongniartii* is planned for spring 2007, one year after the flight.

**Results and discussion**

**Control of the adult beetles**

The results of the spraying of the adult beetles is presented in Figure 1. In the untreated control beetles started to die after 6d and after 10d 92% were dead. λ-Cyhalothrin was the most effective compound; it caused 95 % mortality after two days and 100% after 3d. Interestingly, λ-Cyhalothrin induced a groggy behavior shortly after the treatment, whereas with the other compounds no difference in behaviour was observed. Dimethoat caused > 65% mortality after 2d and 100% mortality after 4d. Azadirachtin and *Bacillus thuringiensis* were less effective compared to Dimethoat and λ-Cyhalothrin. However, after 10d also with these treatments mortality was 100%.

![Figure 1. Mortality of adults of *M. melolontha* after treatment with 4 different insecticides.](image-url)
Controlling the egg deposition
Investigation of *M. melolontha* density in the designated grassland plots to verify the attraction approach are planned for spring 2007. However, preliminary investigations performed on September 5th 2006 demonstrated very high numbers of grubs (up to 200-320 grubs/m²) around the grassland plots. These data indicate that the approach does not sufficiently attract females to the offered grassland plots and by that decrease infestation in the surrounding fields. However, in the whole valley the grassland in the last week of April was very short and therefore, the difference in grass length between the offered plots and the surrounding grassland areas may have been too small to specifically attract the females.

Comparison of the *Melolontha* situation in the Spessart and the Lechgau
The Hessenthal-Mespelbrunn valley is heavily infested by *M. melolontha* grubs which cause severe damage. Additional secondary damage is caused by wild pigs which are digging for the grubs to feed on. As a result the characteristic landscape is negatively influenced by destroyed grassland. Soil can be washed away with heavy rainfalls and flow down the slopes. The population of the cockchafer is furthermore captured in the valley like in a Chinese wok. However, because of the large number of trees around the Hessenthal-Mespelbrunn valley the damage of the adult beetles on the trees is quite low.

Unlike to Hessenthal-Mespelbrunn, the *Melolontha* population in Reichling is not perceived as an agricultural pest by the public or by the farmers. Grubs certainly feed on the roots of plants in the meadows but the damage is negligible. The Lechgau area is supplied better with annual rainfall, the meadows are dominated by dandelion and the soil contains high moisture. And more important, in the Lechgau area there are no wild pigs. The farmers do not have any intention to spray something against grubs. Since decades they tolerate the cockchafers. The damage caused by adults feeding on the trees in Reichling seems to be more severe than in the Spessart because the beetles remain concentrated in a close area of about two kilometres around the village and there is no large forest. Trees in the village become completely defoliated during swarming flights. But due to a second shoot at midsummer, the foliage recovers and the damages nearly disappear. Also garden owners tolerate the beetles. In contrary, more annoying for the people in Reichling are tourists, journalists, and maybe also scientists, who visit Reichling because of this spectacular amount of common cockchafers.

Acknowledgements
We cordially thank Oswald Behl and Hans-Jürgen Wöppel, Office for Agriculture in Würzburg, for their support in the field experiments, Dr Joachim Liebler, Office for Agriculture in Karlstadt/Aschaffenburg, for his help in designing the greens and Kerstin Jung, BBA Darmstadt, for her “If I have time then I always help you in all questions concerning scarabs!”.

References
Spraying adult forest cockchafers with *Beauveria brongniartii*-conidiospores: preliminary results of a large field trial nearby Darmstadt during the main flight in 2006

Kerstin Jung

*BBA, Institute for Biological Control, Heinrichstr. 243, D-64287 Darmstadt, Germany*

**Abstract:** In the vicinity of Darmstadt, 100 ha of forest were treated with an experimental preparation of *Beauveria brongniartii*-conidiospores, applied by helicopter, during the main flight of the forest cockchafer, *Melolontha hippocastani* in 2006. Accompanying research comprised quality control of the experimental preparation, monitoring the fate of *B. brongniartii* after application, observation of the direct effects on beetles as well as monitoring of the effects on the next generation, both in the lab and in the field.

Quality of the conidiospore powder was good in terms of purity, but it contained only half of the anticipated number of viable spores. Therefore, instead of 2x $10^{13}$ spores ha$^{-1}$ only $3.8x10^{12}$ spores ha$^{-1}$ were applied in total. Data on the effect of *Beauveria* on the cockchafer population collected in the field so far are incomplete and premature. However, in the area treated with *B. brongniartii* conidiospore powder, less first instar larvae were found ($24 \pm 19$ m$^{-2}$) compared to the untreated control area ($38 \pm 42$ m$^{-2}$). Conclusive results will be available in 2010 only, when the next beetle generation will emerge.

**Key words:** Entomopathogenic fungi, Hyphomycetes, conidiospores, Scarabaeidae, *Melolontha hippocastani*, helicopter application

**Introduction**

In Germany, the light and sandy soils of the Upper Rhine valley belong to the natural breeding sites of the forest cockchafer, *Melolontha hippocastani*. Population outbreaks in these areas are known from the past, especially in the forests around Darmstadt and Lampertheim (Hessen). Since the mid 1980s again a population increase has been observed in this region (see Zimmermann & Jung, 2004). To date, *M. hippocastani* occurs on approx 12,000 ha forest, alone in Hessen. It endangers forestations on approx 3,000 ha in the most threatened German federal states, Hessen and Baden-Württemberg. Decreasing ground water levels, due to industrial usage, add to the tree-killing factors and served as kind of a bonus for the propagation of the insect, by making conditions more favourable for it.

Earlier, among other measures cockchafer outbreaks were controlled by spraying DDT. Nowadays, no selective and effective insecticide is available, and public concern forbids the use of most broadspectrum insecticides in forests. Since the 19th century, the entomopathogenic fungus, *Beauveria brongniartii* has been recognized as one of the major naturally occurring antagonists of the cockchafer (Giard, 1892). Studying the Lampertheim-population and its limiting factors, Niklas (1960) demonstrated that chemical control measures are not always necessary: In this case, an epizootic of *Ricketsiella melolonthae* led to the decline of the insect population. However, health status of the current cockchafer population in Hessen and Baden-Württemberg does not suggest a potential for a natural breakdown in the near future (Kleespies, pers. commun.).
The use of *B. brongniartii* barley kernel products in soil application against larvae of the field cockchafer, *M. melolontha* is an established and successful plant protection practice e.g. in Switzerland and Austria (u.a. Keller, 2004; Strasser, 2004). In German forests, however, soil application against the larvae of the forest cockchafer is possible in reforestation areas only. Spraying swarming adults with *B. brongniartii*-spore products is an additional option for introduction of *B. brongniartii* into a population. In the late 1980ies this approach was applied successfully against *M. melolontha* (Keller et al., 1997). Here, spraying beetles with blastospores at woodland borders has lead to increased infection rates in the field cockchafer generations following the treatment. In the *M. hippocastani*-infested area a preliminary field test with conidia demonstrated an increase of *B. brongniartii* in the soil following the treatment of beetles feeding in an red oak stand (Jung, 2004).

The Minister of Agriculture of Hessen commissioned the Northwest-German Forestry Research Institute (NW-FVA) with the performance of field trials using various control measures during the main flight in 2006. The present article will focus on the part of these field trials, where *B. brongniartii* was employed. The BBA-Institute for Biological Control was involved in this trial to perform accompanying research and preliminary results are reported herein.

**Material and methods**

*Quality control of Beauveria brongniartii-conidiospore powder*

An experimental preparation of *B. brongniartii* as well as the commercial *B. bassiana*-product Boverol® (95 % Siloxid®, 10^10  conidia/g) was produced by Fytovita (Ostroská Lhota, Czech Republic). The *B. brongniartii* preparation was based on isolate BBA-B.br. 56, which derived from a *M. melolontha*-larva, found at a field in the Lahn-valley (Biskirchen, Germany), where the entomopathogen had established an epizootic. Quality of the preparation was assessed by counting the number of spores and by determination of colony forming units (cfu) on *Beauveria*-selective medium (BSM; Strasser et al., 1996) as well as on non-selective malt-pepton agar (MPA).

*Monitoring the fate of Beauveria brongniartii in the experimental area*

*B. brongniartii* was applied on 9/10 and 17 May 2006 at a rate of 0.47 kg and 50 l water ha⁻¹. The first application on 9 May, could not be completed in one day, due to windy conditions. It was resumed and finished in the afternoon of 10 May. The experimental conidiospore powder was suspended in a tank, using ProNet Alfa (0.15 %; ProAgro GmbH, Abenberg, Germany) as wetting agent and Schaum EXX (SUDAU Agro GmbH, Bockhorn, Germany) as antifoam.

The fate of *B. brongniartii* was investigated on leaves and in soils. On 19 May 2006, leaf samples from deciduous trees (mainly beech and oak) were taken randomly up to a maximum of seven meters from the ground in the treated area. Leaf discs (10 mm Ø) were cut out from each sample using a cork borer. The discs were washed with sterile Tween 80 (0.1 %) and the number of cfu was assessed on BSM. The natural background of *B. brongniartii* in the soils of the experimental area, was assessed from samples collected in April. The second sampling took place in September. Soil samples consisted of 10 separate bore holes each, 20 cm deep, taken with a Pürckhauer drill stick. In both field plots, the *Beauveria*-treated and the untreated control plot, the samples were taken along the transects of digging points, that had been prepared by the forest department for monitoring the insect population. In April, 14 and 10 samples were collected in the treated and untreated area, respectively, whereas in September, the corresponding number of soil samples was 22 and 13, respectively. The soil samples were analysed following a standard procedure for re-isolation of *B. brongniartii* spores from soil (Jung, 2004).
Effects of spraying Beauveria: Mycosis of beetles and effects on the offspring in the lab
On 11 and 12 May 2006, beetles were collected in the treated and untreated forest areas. They were held in the experimental garden of the BBA-institute in a) terrariums (40x25x25 cm lxwxh; 7:5 females:males each; n=12), b) plastic pots (65 l, 58 cm Ø; 15:10 females:males each; n=10), and c) flight cages (300x300x200 cm, lxwxh; 100:50 females:males each; n=4). The terrariums and plastic pots were filled with sand and standard potting soil (Fruhstorfer Erde, Typ Null) to allow the females to bury and lay eggs. Here, the beetles were fed with fresh leaves of red oak. In the flight cages, potted deciduous trees (30-40 cm/40-50 cm; German oak, basswood and maple), were placed (8-11 per cage), in order to provide food and soil for egglaying. The bottom of the flightcages was covered with nets to prevent the females from egglaying in the ground below. After all beetles were dead, the ones laying on the surface of every container, were collected and transferred into moist chambers to allow a possible fungal infection to become visible. Starting in September, the soil of the terrariums, pots and the potted trees was sifted for the offspring and the beetles that had been buried. Living offspring was held in moist soil. Dead specimen were incubated in moist chambers.

Effects of spraying Beauveria: Monitoring of the insect population and its health in the field
In April, before emergence from soils, the density of beetles was assessed by the NW-FVA by digging holes (154 and 146 in the treated and untreated area, respectively). The holes were arranged in grids of 50 x 50 meters through the areas and durably marked. The same sites were checked for the next generation in September. A part of the total number of white grubs from both the untreated and the treated area were brought to the BBA-institute and kept in plastic pots (5 cm Ø) in moist standard potting soil (Fruhstorfer Erde, Typ “Null”) in an incubator at 20 °C for up to six weeks. They were fed with carrot slices and checked weekly. Dead specimen were either examined directly or transferred into moist chambers.

Results and discussion

Quality control of Beauveria brongniartii-conidiospore powder
Productivity of the isolate BBA-B.br. 56 was not very good: It produced 10times less spores per squaremeter, when the company studied it in comparison with their commercial Boverol® (B. bassiana) isolate (Nesrsta, pers. commun.).

The experimental preparation was send in three different shipments, consisting of different batches. A pre-application sample was received in March. This pre-sample, as well as the first batch (1st shipment; used entirely at the first application date), fullfilled the technical product data in terms of number of spores (claimed spore concentration 1x10^10 spores g^-1; Table 1). However, not all of them were viable: The number of viable spores was reduced by a factor 2-10 (Table 1). The three other batches (2nd and 3rd shipment; used at the first and second application date did not fulfull the expectations: The countable number of spores was only half the expected value. and again not all of them were able to germinate and grow: The number of colonies was less than the number of spores counted in the microscope. Comparison between the number of cfu on BSM and on MPA showed no significant difference between the two media. Moreover, no contamination could be detected by these methods.
Table 1. Number of spores \([g^{-1}]\) and viable spores assessed as colony forming units (cfu) on *Beauveria*-selective medium (BSM) and on non-selective malt-pepton agar (MPA). Values are the mean of 3 replicates ± standard deviation. n.a. = not assessed.

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>spore count ([g^{-1}])</th>
<th>cfu ([g^{-1}]) on BSM</th>
<th>cfu ([g^{-1}]) on MPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre-sample</td>
<td>(1.8 \times 10^{10})</td>
<td>(2.2 \times 10^9 \pm 2.6 \times 10^8)</td>
<td>n.a.</td>
</tr>
<tr>
<td>03620616</td>
<td>(1.0 \times 10^{10})</td>
<td>(3.0 \times 10^9 \pm 2.1 \times 10^9)</td>
<td>n.a.</td>
</tr>
<tr>
<td>04620617</td>
<td>(6.3 \times 10^9)</td>
<td>(5.9 \times 10^9 \pm 1.5 \times 10^9)</td>
<td>(5.2 \times 10^9 \pm 4.2 \times 10^8)</td>
</tr>
<tr>
<td>05620618</td>
<td>(5.3 \times 10^9)</td>
<td>(3.4 \times 10^9 \pm 1.7 \times 10^9)</td>
<td>(4.4 \times 10^9 \pm 1.3 \times 10^9)</td>
</tr>
<tr>
<td>06620618</td>
<td>(6.5 \times 10^9)</td>
<td>(3.9 \times 10^9 \pm 1.3 \times 10^9)</td>
<td>(4.0 \times 10^9 \pm 1.3 \times 10^9)</td>
</tr>
</tbody>
</table>

**Monitoring the fate of Beauveria brongniartii in the experimental area**

Based on the number of cfu on BSM, the total application rate was calculated as \(3.8 \times 10^{12}\) spores ha\(^{-1}\) (2x 0.47 kg ha\(^{-1}\); mean of \(4 \times 10^9\) cfu \(g^{-1}\) for the four batches).

Numbers of spores detected on the leaves of deciduous trees from the treated area were very variable (Table 2). \(3.9 \times 10^3\) viable spores cm\(^{-2}\) leaf area was the highest number of spores recorded. It derived from a heights of 4 m. The averaged number of viable spores above 4 m was less than below 4 m (314 compared to 1070, respectively; Table 2). One reason could be, that at a greater heights spores are more exposed to sunlight and remain viable for shorter periods only. The highest number of spores recorded is still 10times less than the possible maximum of \(3.8 \times 10^4\) cm\(^{-2}\) based on the total application rate of \(3.8 \times 10^{12}\) spores ha\(^{-1}\). However, taking into account the mostly warm and sunny weather between the two application dates, this is regarded as a good result, in terms of a targeted application (that means, adult beetles were hit, because they mainly lingered on the leaves of smaller deciduous trees before dawn) and persistence of spores on the leaf surface.

Table 2. Number of viable *Beauveria brongniartii*-spores [cfu cm\(^{-2}\) leaf area] on deciduous tree leaves, sampled 2 days after the second application. Values are the mean of 3 replicates ± standard deviation.

<table>
<thead>
<tr>
<th>heights [m]</th>
<th>cfu cm(^{-2}) leaf area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(2728 \pm 1040)</td>
</tr>
<tr>
<td>1</td>
<td>(54 \pm 11)</td>
</tr>
<tr>
<td>2</td>
<td>(382 \pm 127)</td>
</tr>
<tr>
<td>2</td>
<td>(1116 \pm 442)</td>
</tr>
<tr>
<td>4</td>
<td>(499 \pm 334)</td>
</tr>
<tr>
<td>4</td>
<td>(1246 \pm 621)</td>
</tr>
<tr>
<td>4</td>
<td>(3901 \pm 3242)</td>
</tr>
<tr>
<td>4.5</td>
<td>(161 \pm 175)</td>
</tr>
<tr>
<td>5</td>
<td>(884 \pm 617)</td>
</tr>
<tr>
<td>5.5</td>
<td>(85 \pm 112)</td>
</tr>
<tr>
<td>6.5</td>
<td>(71 \pm 32)</td>
</tr>
<tr>
<td>6.5</td>
<td>(368 \pm 138)</td>
</tr>
</tbody>
</table>
No *B. brongniartii* was found in soil samples that had been taken before the application. Analysis of soil samples taken in September is in progress. The ostensible absence of the pathogen in the soils of the experimental area could explain the good health of the cockchafer population.

**Effects of spraying Beauveria: Mycosis of beetles and effects on the offspring in the lab**

Between 55 and 67 % of the cockchafer were recovered from the surface of the different containers in which they had been held (Table 3). No clear effect of the treatment was measurable, because 19-26 % of the cockchafers collected from the untreated container were found to be mycosed by *B. brongniartii* as well. However, the highest rate of mycosis was recorded in the specimen that had been collected in the treated area and were held in pots (Table 3). Analysis of the data from the soil of all containers is still ongoing, therefore no results can be presented here.

**Effects of spraying Beauveria: Monitoring of the insect population and its health in the field**

The mean number of adult cockchafer was 8 ± 9 and 14 ± 16 m⁻² in the untreated and the *B. brongniartii*-treated area, respectively, estimated from the data collected before the application. In September, the mean number of larvae m⁻² was 38 ± 42 and 24 ± 19 m⁻² in the untreated and the *B. brongniartii*-treated area, respectively. Since these numbers are based on an incomplete dataset – they represent the number of white grubs from 67 and 46 holes out of 140 and 154, respectively, for the untreated and the *Beauveria*-treated area – they have to be considered as preliminary. Nevertheless, the smaller number of first instar larvae found at the *Beauveria*-treated area, which started off with a higher number of adult beetles, suggest a positive control effect of the *Beauveria* applications. Observation of the collected white grubs is still ongoing.

| Table 3. Number of cockchafer recovered from the surface of the containers, and percentage based on the total number of cockchafer (144, 250 and 600 in terrariums, pots and field cages, respectively), together with the number and percentages of mycosed specimen out of the recovered ones. See text for more details. |
|---|---|---|---|---|
| | untreated | treated | | |
| | recovered | mycosed | recovered | mycosed |
| Terrarium | 87 | 23 | 97 | 30 |
| | (60 %) | (26 %) | (67 %) | (31 %) |
| Pot | 138 | 27 | 153 | 65 |
| | (55 %) | (19 %) | (61 %) | (42 %) |
| Field cage | 359 | 76 | 356 | 66 |
| | (60 %) | (21 %) | (59 %) | (18 %) |

**Preliminary Conclusions**

Based on the observations and data collected so far, the following conclusions are drawn:

- From a technical point of view, the experimental conidiospore powder of *B. brongniartii* was successfully applied by use of a helicopter to approx. 100 ha forest area, heavily invested with *M. hippocastani*. 
• Instead of $2 \times 10^{13}$ spores/ha only $3.8 \times 10^{12}$ spores/ha were applied. The reduced application rate was in part due to the use of an isolate of *B. brongniartii*, which had not been selected for good spore production.

• Nevertheless, two days after the 2nd application, up to $4 \times 10^3$ viable spores/cm² were recovered from leaves within the treated area.

• Direct effects on the beetles could not be demonstrated in the lab due to a relative high rate of mycosis in beetles from the untreated area. This is a phenomenon often observed in trials with cockchafers and *B. brongniartii* (Keller, pers. commun.; Zimmermann & Jung, unpubl.).

• Although the set of field data is still incomplete, the reduced number of first instar larvae found in the *Beauveria*-treated area is interpreted as a positive treatment effect.

**Acknowledgements**

The research is financed by the Hesse Ministry of Environment, Agriculture and Consumer Protection (HMULV). The good cooperation with both, the Northwest-German Forestry Research Institute (NW-FVA) and the forest department of Darmstadt, as well as the assistance of numerous co-workers is gratefully acknowledged.

**References**


White grub control in golf courses

Siegfried Keller, Christian Schweizer
Agroscope Reckenholz-Tänikon Research Station ART, Reckenholzstrasse 191, CH-8046 Zürich, Switzerland

Abstract: A first white grub damage in a golf course in Switzerland was reported in the year 2000. Since then the number of severely damaged golf courses increased to six. In addition to that numerous damages were reported from sport and school areas as well as from airfields. Damages in golf courses are all located in alpine valleys and were done either by *Amphimallon solstitiale*, *A. majale*, *Phyllopertha horticola* or *Melolontha melolontha* or by a variable association of these species. Depending on the pest species present we applied *Metarhizium anisopliae* alone or in combination with *Beauveria brongniartii*. The application was done with a commercial drill machine, exceptionally with a rotary machine. The results achieved so far show that the fungi established in the soil and reduced of the white grub populations so that no more damages were visible.

Key words: Scarabaeidae, white grubs, microbial control, entomopathogenic fungi, *Metarhizium anisopliae*.

Introduction

In the past years an increasing number of damages in golf courses, sport fields, airfields and also meadows were reported. They all originate from alpine valleys at altitudes between 600 and 1400 m. Most damages were done by *Phyllopertha horticola*, sometimes by *Amphimallon solstitiale*, *A. majale*, and occasionally by *Melolontha melolontha*. Some golf courses had to be closed due to the heavy damages originally done by white grubs but aggravated by the searching activities of predators like crows and badgers.

Damages done by *A. solstitiale* in lawns have been well known for long time, also damages done by *M. melolontha*. In contrast to that, *A. majale* was only known by entomologists and damages have never been reported. Also, *P. horticola* has not been reported as pest species until a few years ago. This species seems to become more and more important as pest species, also in meadows.

Before the greenkeepers contacted us they have applied chemical insecticides and nematodes but the results were unsatisfying. We concentrated our efforts on the control of *Amphimallon* spp. and *P. horticola* with the fungus *Metarhizium anisopliae*. We selected virulent isolates and produced them with the same methods as used for the production of *Beauveria brongniartii*.

Material and Methods

In most golf courses usually the whole area was treated, sometimes only the area with high white grub densities (Tab. 1). At least a surface of 20 m x 20 m was left untreated. The fungus material was applied with a commercial drill machine, exceptionally with a rotary machine (Brigels). White grub and soil samples were taken from most golf courses in spring and autumn in this untreated area and in an adjacent treated area of the same surface. In addition
to that further samples were taken from the treated fairways. The soil samples were processed in order to obtain the fungus densities expressed as colony forming units (cfu) per gram fresh soil.

At Alvaneu four white grub samples on a surface of \(\frac{1}{4}\) m\(^2\) and ten soil samples were taken at each sampling date. The same procedure was chosen for the commercially treated courses at Brigels and Engelberg.

At Grindelwald a trial with a complete randomised block design was carried out with plots measuring 20 m x 20 m. Three treatments were applied: 1. untreated; 2. treated with 40 kg product per ha, and 3. treated with 80 kg product per ha. Two samples of \(\frac{1}{4}\) m\(^2\) per plot were examined for grub densities and infection rates and 10 soil samples were taken before the application and 4, 6 and 13 months after the application.

At Domat/Ems a plot each of 20 m x 20 m was treated with \(B. \) brongniartii, \(M. \) anisopliae or a mixture of these fungi at a rate of 40 kg/ha each. A fourth plot was left untreated. Half of the fairways were treated with \(M. \) anisopliae alone and the other half with a mixture of \(B. \) brongniartii and \(M. \) anisopliae with the same amount of product.

### Table 1. Overview on the golf courses treated with entomopathogenic fungi to control larvae of different scarab species.

<table>
<thead>
<tr>
<th>Location and altitude</th>
<th>Trial design</th>
<th>Treatment</th>
<th>Pest species present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alvaneu-Bad 950 m</td>
<td>Single plot</td>
<td>25.8.2000, 50 kg/ha, (M. ) anisopliae</td>
<td>(P. ) horticola, some (A. ) majale</td>
</tr>
<tr>
<td>Grindelwald 980 m</td>
<td>Randomised block design, 4 replicates</td>
<td>27.4.2003, 40 kg/ha, 80 kg/ha, (M. ) anisopliae</td>
<td>(P. ) horticola, some (A. ) majale</td>
</tr>
<tr>
<td>Brigels 1300 m</td>
<td>Single plots, treated fairways</td>
<td>18.8.-26.9.2005, application with rotary machine</td>
<td>(P. ) horticola</td>
</tr>
<tr>
<td>Vulpera 1300 m</td>
<td>Partially treated fairways</td>
<td>End April 2005, 40 kg/ha, (M. ) anisopliae</td>
<td>(P. ) horticola</td>
</tr>
<tr>
<td>Domat/Ems 600 m</td>
<td>Single plots, treated fairways</td>
<td>19.4.2006, 40 kg/ha, (M. ) anisopliae, (B. ) brongniartii</td>
<td>(M. ) melolontha, (P. ) horticola, (A. ) solstitialis</td>
</tr>
<tr>
<td>Engelberg 1000 m</td>
<td>Treated fairways, untreated plot</td>
<td>26.4.2006, 40 kg/ha, (M. ) anisopliae</td>
<td>(P. ) horticola</td>
</tr>
<tr>
<td>Lenzerheide 1400</td>
<td>Treated fairways, untreated plot</td>
<td>26.4.2006, 40 kg/ha, (M. ) anisopliae</td>
<td>(P. ) horticola</td>
</tr>
</tbody>
</table>

### Results

The first trial to control white grubs on golf courses was carried out at Alvaneu. Three months after the treatment the densities were moderate but the disease had already well established in the treated plot; 77% of the collected larvae succumbed to \(M. \) anisopliae (Tab. 2). In the following year, the only larva found was infected. In the untreated plot the density increased from November 2000 till September 2003 by a factor of 7.1 to 233 grubs/m\(^2\), while it decreased slightly in the treated plot by a factor of 0.8.
Table 2. Development of *P. horticola*-populations and of infections due to *M. anisopliae* at Alvaneu.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Density untreated (ind/m²)</td>
<td></td>
<td>33</td>
<td>59</td>
<td>57</td>
<td>233</td>
</tr>
<tr>
<td>Density treated (ind/m²)</td>
<td></td>
<td>59</td>
<td>3</td>
<td>49</td>
<td>47</td>
</tr>
<tr>
<td>Mycosed grubs untreated (%)</td>
<td></td>
<td>14</td>
<td>2</td>
<td>no data</td>
<td>no data</td>
</tr>
<tr>
<td>Mycosed grubs treated (%)</td>
<td></td>
<td>77 (100)</td>
<td>no data</td>
<td>no data</td>
<td>no data</td>
</tr>
</tbody>
</table>

At Grindelwald the densities in 1/4 m² four months after treatment were 12.6 larvae (STD = 15.4) in the untreated plots and 14.75 (STD = 17.9) and 24.63 (STD = 21.2) in the plots treated with 40 kg/ha and 80 kg/ha respectively. The densities in the treated plots decreased in the following two months to 27% and 21% of the previous density respectively, but the reduction was statistically significant only for the higher dose (p = 0.024) (Fig. 1). Thirteen months after the treatment also the untreated population decreased, the reduction of the population treated with the higher dose was still significant (p = 0.019). At that time 54% (40 kg/ha) and 44% (80 kg/ha) of the white grubs succumbed to the *M. anisopliae* (Fig. 1).

Before the treatment *M. anisopliae* was present at densities below 200 cfu/g soil (Fig. 2). In the untreated plots the fungus densities remained low while they increased in the treated plots. Six months after the treatment they reached densities of about 2000 cfu/g soil for either application rate. Thirteen months after the treatment the fungus density in the plots treated with the lower dose had decreased significantly, those treated with the higher dose had increased to about 3000 cfu/g soil (Fig. 2). This different development of the fungus populations is not reflected by the observed infection rates.
The data on fungus development obtained at Domat/Ems are given elsewhere (Keller et al., this Bulletin). Data on white grub development are not yet available. The data from the other sites are not yet processed.

![Figure 2. Development of M. anisopliae at Grindelwald in untreated plots and in plots treated with 40 or 80 kg product per ha.](image)

**Discussion**

White grub damages in golf courses and other places with lawns are increasing in Switzerland. Most damages are done by *P. horticola*, sometimes in associations with *Amphimallon* spp and/or *M. melolontha*. The reasons for that could be an adaptation especially of *P. horticola* to this relatively new and more and more available habitat or the global climatic change. The development of resistance to insecticides is very unlikely. Some of the involved greenkeepers had used insecticides but only for a short time until they realised that they could not control successfully the white grubs. This missing success was one of the reasons why they asked us for trials with entomopathogenic fungi. The other reason was the increasing demand for environmentally friendly control methods.

The results of our investigations demonstrated that white grubs others than *M. melolontha* can successfully and sustainably be controlled with *M. anisopliae*. The only problem for the greenkeepers is the way of application, which leads to slits in the ground. However, when the fungus is applied in spring the slits are no more visible when the main golf season starts. The results achieved in these trials are part of a dossier submitted for the registration of the used product. Official registration is expected for end 2006.

**Acknowledgements**

We thank the greenkeepers of the involved golf courses for their support and the team of E. Schweizer Seeds SA and for their help with the samplings.
Entomopathogenic nematodes and target soil insect pests in tree habitats in the Czech Republic, with focus on sawflies and cockchafers

Vladimír Půža1,2, Zdeněk Mráček2, Jaroslav Holuša3

1Faculty of Biological Sciences, University of Southern Bohemia, Branisovska 31, Ceske Budejovice, Czech Republic; 2Institute of Entomology, BC AVCR, Branisovska 31, Ceske Budejovice, Czech Republic; 3Forestry and Game Management Research Institute Jiloviste-Strnady, Czech Republic

Abstract A long-term survey performed in the Czech Republic shows that EPNs naturally occur in the majority of tree habitats and have therefore a potential to control resident insect pests. One of the most important target insect pests in tree habitats are sawflies and cockchafers.

A study focused on EPNs and web spinning larch sawfly (Cephalcia lariciphila) was started in the larch-spruce forest in the Czechomoravian high lands. Two sites – one with an outbreak of the sawfly and one control site with a sporadic incidence of C. lariciphila were chosen for the sampling. In spring and autumn 2005 and 2006 the sites were examined for the presence and abundance of EPNs and for the abundance and composition of the soil insect community. At the site with the sawfly outbreak a very abundant population of EPNs (approx. 30 000 infective juveniles per m²) was detected, while the population of EPNs at the control site was more than ten times lower. Both sites are inhabited by a variety of potential insect hosts of EPNs (mainly dipterans: Sciaridae, Dolichopodidae, Empididae, and coleopterans: Cantharidae, Carabidae) on which S. kraussei probably survive periods with low density of C. lariciphila larvae in the soil. Naturally nematode infected Cephalcia larvae and newly hatched adults were found during the study. Natural parasitisation attained 20%.

In spring 2006, a site with a serious outbreak of M. hippocastani near Lipnik, Central Bohemia, was thoroughly surveyed for EPNs. Four steinernematid species (Steinernema kraussei, S. silvaticum, S. intermedium and S. feltiae) were isolated from the area. These nematodes will be tested for pathogenicity towards Melolontha larvae.

Key words: Steinernema, forest pests, Cephalcia, Melolontha

Introduction

Entomopathogenic nematodes (EPNs) are lethal parasites of various soil-dwelling insects and are being used for the biological control of many insect pests. However, until now, little attention was devoted to the research of the potential of EPNs to control the insect pests in forest habitats.

Long term sampling in the Czech Republic (Mráček et al, 1999; Mráček and Půža, unpublished data) revealed a total of 5 EPN species in more than one half (55%) of tree habitats. The dominant species were Steinernema kraussei occurring in 50% of EPN positive sites and S. feltiae with 25% occurrence. Among the forest habitats, larch and oak stands have the highest occurrence of EPNs (87 and 76% respectively). Generally, sites with an obvious insect outbreak were very often inhabited by EPNs (88% of the sites were EPN positive).

Insects with strong population outbreaks in the Czech forests are sawflies, cockchafers, bibionid flies and various lepidopterans. Among these insects sawflies and cockchafers seem to be suitable target pests for EPNs.
In the present paper a study of the role of EPNs during an outbreak of the larch web spinning sawfly (Cephalcia lariciphila) and the results of a survey of the EPN occurrence at the site with an outbreak of the chestnut cockchafer (Melolontha hippocastani) are presented.

**Material and methods**

An outbreak of C. lariciphila
Investigations were performed in a larch-spruce forest in Czechomoravian highlands, where an outbreak of C. lariciphila started in 2000. Two sites in the forest were chosen for the study: a site with an outbreak and a control site with no sawflies. Soil samples for the estimate of EPN abundance and of the quantity and quality of the soil insect community were taken in the spring and autumn of 2005 and 2006.

EPNs were extracted using the Galleria baiting method (Bedding and Akhurst, 1975). Death Galleria larvae were dissected, adult nematodes were counted and the nematode numbers were converted into abundance of EPNs per square meter.

C. lariciphila larvae were hand-picked from the samples while other soil insects were extracted using Tullgren funnels and identified to the family level.

Sampling for EPN distribution was performed to determine if the EPN distribution reflects the distribution of C. lariciphila, which is present mainly under larch canopy. Samples were taken in the summer 2006 at each site under the canopy of each of 10 randomly chosen larch and spruce trees.

The natural parasitization of C. lariciphila by EPNs was assessed in the spring 2006 by hand-picking C. lariciphila from 0.5 x 0.5m samples and expressed as a percentage of infected individuals.

An outbreak of M. hippocastani
A site with an outbreak of M. hippocastani was investigated in spring 2006 near Lipnik in Central Bohemia. Samples were taken at the outbreak site and in surrounding oak forests.

**Results**

An outbreak of C. lariciphila
EPN species Steinernema kraussei and S. feltiae were found at both sites. Mean EPN density at the outbreak site was approximately 40 000 IJs per m² while at the control site, EPN density was markedly lower (5000 IJs per m²) (Fig.1). The densities of C. lariciphila larvae at the outbreak site are shown in the Figure 1. The insect community at both sites was similar both in quality and quantity. The most common insect hosts were fly larvae (Sciaridae, Dolichopodidae, Empididae) and beetle larvae (Cantharidae, Carabidae).

At the outbreak site, EPN density was significantly higher (t=-5.39; p<0.000) under canopies of larch while in the control site, the EPNs density did not differ between larch and spruce canopies (Z=-1.06; p=0.288).

The immediate parasitisation of Cephalcia larvae by EPNs was ca 20%. During the survey several infected adults were found.

An outbreak of M. hippocastani
EPN species Steinernema kraussei and S. silvicatum were found at the outbreak site at a density of about 1000 IJs per m². Surrounding oak forests host S. silvicatum, S. intermedium and S. feltiae at a density attaining 20 000 IJs per m².
Discussion

An outbreak of *C. lariciphila*

The markedly higher density of EPNs at the outbreak site was probably due to the high density of *C. lariciphila* larvae because sawfly larvae are suitable hosts for EPNs. However, as can be seen in Figure 1, the outbreak of *C. lariciphila* is presently declining and therefore it is possible that the EPN density at the outbreak site will decrease in the future.

The EPN distribution obviously reflected the distribution of *C. lariciphila* because at the outbreak site EPN abundance was much higher under the canopy of larch trees, while at the control site there were no differences between EPN density under the canopy of larch and spruce trees.

Natural parasitisation of 20% is very high and EPNs seem to be an important factor to naturally decrease a population of *C. lariciphila*. We assume that at our site, EPNs significantly contributed to the decline of the *C. lariciphila* outbreak.

An outbreak of *M. hippocastani*

We suppose that the low density of EPNs at the site with the outbreak might be due to use of several pesticides. All EPN species were established in laboratory cultures and will be tested for their pathogenicity towards cockchafer larvae in order to find the most effective species for the biological control of white grubs.

References


A great increase of population of Common Cockhafer (*Melolontha melolontha* L.) in Idrija region in Slovenia

Anka Poženel, Mojca Rot
*Agriculture and Forestry Institut Nova Gorica, Pri hrastu 18, SI-5000 Nova Gorica, Slovenia*

**Abstract:** During 2002 and 2006 a great increase of population of Common Cockhafer (*Melolontha melolontha* L.) was observed in the Idrija region in Slovenia. In 2002 the third larval stage of cockchafer with an average of 100 grubs per m² completely damaged 370 ha of grasslands. In 2004 damage was caused by adult cockchafer. After egg deposition the population increased to 200 grubs per m². Grass was damaged up to 50 % by the grubs of the first larval stage. In 2005 an average of 226 grubs per m² was observed in the region. 760 ha of grasslands were damaged, which represents 62% of all agricultural land in the region. Different methods (mechanical, biological and chemical treatments) were used to reduce the population of the pest, but they were only partly successful. In June 2005 92 ha of grasslands were treated with *Beauveria brongniartii*. The efficiency of *Beauveria* was 38.7 %. The total decrease in the number of grubs in the treated area was 88 %.

**Keywords:** *Melolontha melolontha*, white grubs, *Beauveria brongniartii*, damaged grasslands, Slovenia

**Introduction**

An outbreak of the Common Cockchafer (*Melolontha melolontha* L.) was observed in the western part of Slovenia in Idrija region in 2002. Highest damages occurred in grasslands between villages Zadlog in Idrijski log. The main characteristics of the landscape in this region are meadows and pastures spread on karsts plateau at altitudes between 650 and 750 m which are surrounded by forests. The soil formed on limestone basis is shallow and stony with large permeability. The underground drainage supplies the water catchments area which is the most important and the only source of drinking water for the Idrija city. The area belongs to the water protection zone and the land use is regulated.

The most important agricultural branch in the region is cattle-breeding; almost all the farms are orientated in milk production. Some of them are also organic farms. The whole area has a great potential for development of organic farming in the future.

*Melolontha* is a frequent pest of Slovenian meadows and pastures, especially those overgrown with *Taraxacum officinale* L. In the last 50 years there was no economical important damage in Slovenia caused by this pest. But in 30s and 50s of the previous century a great damage by *Melolontha* populations was reported in the Idrija region and other regions in Slovenia. The outbreaks of cockchafers were observed in years 1932 and 1935. In 1953 the control of adult cockchafers was done by shaking the trees, picking up the insects and boiling them. Finally, the population was reduced by late frost and snow in the spring 1956.

The *Melolontha* population in Slovenia has a three year life cycle with different flight regimes in different location (Janežič, 1958; Vrabl, 1992). In Idrija region the adults flew in the years 2001 and 2004. In 2007 another flight is expected.

In 2002 the third larval stage of cockchafer with an average of 100 grubs per m² completely destroyed 370 ha of grassland (Poženel, 2005). In 2004 damage was caused by
adult cockchafer. After egg deposition the population increased to more than 200 grubs per m² and consequently the grass was damaged up to 50 % by the grubs of the first larval stage. In 2005 an average of 226 grubs per m² was observed in region and 760 ha of grasslands were damaged. In 2005 an economic damage caused by *M. melolontha* was noticed on 1000 ha of agriculture land in the whole country.

Since 2002 a lot of work has been done by farmers and experts who tried to find a solution for controlling the pest. Different methods (mechanical, biological and chemical treatments) were tested in order to reduce the population of the pest, but they were only partly efficient. The use of rotary hoes for a mechanical control of the pest was the most efficient method, but not the best, because of lost of harvest. To find out a long-term and suitable solution for this environmental sensitive area we decided to test biological control with *Beauveria brogniartii* (Sacc.) Petch, 1924.

**Material and method**

*Beauveria brogniartii* (Sacc.) Petch, 1924 was applied in the June 2005. This was the first trial carried out in Slovenia with the entomopathogenic fungus. Experiences with this biocontrol agent were adapted and used from Switzerland, Austria and Germany (Keller, Brenner, 2005; Benker, Leuprecht, 2005)

The *Beauveria* fungus was applied in the form of MELOCONT®-Pilzgerste. The incorporation of MELOCONT® in the turf was done by use of a special seedling machine on 14th June 2005. At the time of application third instar larvae (shortly after moulting) were located in the 2-3 cm top soil layer of the turf. The soil was moist, after application a period of three weeks without rain and daily temperatures up to 25°C followed. During the summer the level of soil moisture was sufficient. The whole treated area was 90 ha.

The number of grubs was counted at 5 sites, of 0.5 ha each. At each site the number of grubs was counted at 10 places of ¼ square meters. The sod was dug up and the soil was searched for grubs. The untreated plots were assessed in the same way. Monitoring was done on July 21 2005, October 18 2005, May 15 2006 and on July 19 2006.

**Results and discussion**

The number of grubs of *M. melolontha* L. in grasslands treated with *B. brongniartii* was reduced by 88.2 % during the trial (Figure 1). Four weeks after the treatment (July 2, 2005) the decrease was only 29 %, which was not enough to prevent the damage. The turf was ruined. No *Beauveria* infected grubs were found at that time.

In untreated plots the number of grubs was reduced by 49.5 % during the trial (Figure 1). The main reason for reduction was the flood at the beginning of October 2005. The grasslands were covered with water and the grubs came out on the surface. After water run off they were killed by ultraviolet radiation of the sunlight, birds and other predators. In the autumn the number of *Beauveria* infected grubs increased to 4.4 grubs per m².
In the spring 2006 the number of grubs in the treated area increased compared to the number counting in autumn. However, a decrease in the number of grubs was observed at last counting on of July 19, 2006 (Figure 1).

The total decrease in number of grubs was 88 % by comparison with the starting number. The results are encouraging for use of the biological control method and comparable with results of previous trails (Benker, Leuprecht, 2005). Calculated efficiency of \textit{B. brongniartii} was 38.7 %. Reasons for the rather low efficacy of \textit{B. brongniartii} could be a late application and the unfavourable soil conditions after the treatment. In the treated area the soil is shallow and after the fungus application there was no rain for three weeks, which may have limited the activity of \textit{B. brongniartii} right after the treatment.

In this sensitive environment with special characteristic of the soil including the water protecting zone a chemical control of \textit{M. melolontha} L. is not acceptable. Results presented demonstrate that biological control with \textit{B. brongniartii} may provide a good solution. In the future we will have to incorporate the fungus in the soil early in spring, when the moisture is sufficient to improve the efficiency of \textit{B. brongniartii}. For the region discussed herein this would be end of April.

**Acknowledgements**

We thank Dr. Herman Strasser and Dr. Barbara Pernfuss for technical support and Roberto Kron Morelli from Agrifutura s.r.l., Italy.

**References:**


Miscellaneous
Tipula paludosa population dynamics: challenging the myth of environmental limitation

Rod P. Blackshaw¹, Sergei V. Petrovskii²
¹School of Biological Sciences, University of Plymouth, Drake Circus, Plymouth PL4 8AA, UK; ²Department of Mathematics, University of Leicester, Leicester, LE1 7RH, UK.

Abstract: The larvae of Tipula paludosa – leatherjackets - are well known pests of grassland and cereals in northwest Europe and are also now becoming a problem in North America. It has become widely accepted that low rainfall at the time of egg and first instar reduces populations, and conversely, wet conditions at this time promote numbers. A meta-analysis of survey data shows there is substantial evidence for negative feedback between growth rate and population size irrespective of local conditions. This relationship is quantified and used to develop a simulation model that can accommodate different regional population dynamics.

Key words: Tipula paludosa, population limitation, population regulation, meta-analysis

Introduction

Leatherjackets, the larvae of Tipula paludosa Mg. have been reported as pests in cool temperate regions of north-west Europe (see Blackshaw and Coll 1999 for a review), and have successfully invaded and colonised similar climatic regions of north-western USA where they are currently spreading (Umble and Rao 2004). They are historically associated with damage to spring cereals that follow grass. Damage to grassland also occurs in both reseeds and established swards but the majority is to long term leys and pastures (Blackshaw 1985). He estimated that leatherjackets were responsible for in excess of £15m worth of damage in grassland in Northern Ireland alone each year.

Whilst damage from these pests can be readily controlled with insecticides (Blackshaw and Coll, 1999) they are re-emerging as pests with the reversion to more sustainable farming systems, such as organic, with its increased reliance on grass-based fertility building rotations. In these circumstances, management interventions will require a greater understanding of the pest’s ecology. In this paper we address the issue of whether T. paludosa populations are limited by adverse environmental conditions (Milne et al. 1965; Mayor and Davies 1976) or are regulated in the sense of Berryman et al. (2002). We present a meta-analysis of annual leatherjacket survey data from UK regions and show how this can be used to develop a parameter-sparse simulation model of T. paludosa population dynamics.

Material and methods

Published data exist for annual leatherjacket surveys in grassland for Northern Ireland from 1970 to 1988 (Blackshaw & Perry 1994), south-west England, separable into six counties, from 1963 to 1974 (Mayor & Davies, 1976), and a time-series can be recreated for northern England from 1948 to 1963 (Cohen 1953; White 1963). These data exist as mean counts. In addition, unpublished data are available for south-west Scotland from 1975 to 1994, and north-east Scotland for 17 years from 1987/88 to 2003/04 as individual field counts; annual
means were calculated to provide similar data to the other surveys. There is some uncertainty surrounding the identification of leatherjackets with possible confusion between \textit{T. paludosa} and \textit{Tipula oleracea} L.. However, Humphreys et al. (1993) showed that the larvae of \textit{T. oleracea} were not commonly found in agricultural grassland in northern Britain. Thus for the purposes of this paper we can consider counts of leatherjackets from annual grassland surveys to be \textit{T. paludosa}.

The annual survey mean counts were normalised as abundances relative to the regional mean from each source time series. From these, the annual per capita increase was calculated, and regressed against the relative abundance for four time lags. These relationships were then used to create a general population model. The data for north-east Scotland were omitted so that they could be used to test this simulation model.

\textbf{Results and Discussion}

The regressions of log(per capita increase +1) against log(relative abundance) yielded $r^2$ values of 0.343, 0.177, 0.016 and 0.047 for time lags of 1 to 4 respectively. Further analysis was therefore restricted to a time lag of one, where the per capita rate of increase/relative abundance data showed strong evidence of negative feedback (Figure 1).

![Figure 1. Per capita growth rate and relative abundance for leatherjacket survey data. Two separate but parallel relationships are apparent; lower points (■) concur with population crashes and their associated resurgences (▲) form the extreme of the relationship that occurs under normal rainfall conditions (●).](image)

The data points in Figure 1 separated into two discernable relationships so they were analysed independently. The resultant regressions were log(per capita increase +1) = -0.0252 - 0.8135.log(relative abundance) ($r^2 = 0.688$), and log(per capita increase +1) = -2.046 - 0.9236.log(relative abundance) ($r^2 = 0.959$) for the upper and lower data points respectively. There was no significant difference between the slopes ($t=1.258; P>0.05$) and so a weighted average slope of 0.822 was calculated. These two regression lines demonstrate similar
density-dependent responses under different conditions. The lower (and smaller) data set consists of growth rates where there is a rapid population decline or ‘crash’ in the sense of Milne et al. (1965).

This regression analysis leads to a model in the form corresponding to a generalised logistic population growth:

$$
\Delta N_t = N_t \left(10^{\alpha} \left[ \frac{N_t}{E[N]} \right]^{-\beta} - 1 \right)
$$

where $N_t$ is the average population size at a given year, $\Delta N_t = N_{t+1} - N_t$ is the increase in number over the consequent season, $E[N]$ is the mean population size, $\alpha$ is the intercept and $(-\beta)$ is the slope. Stochastic error is included by the term $r$, $E[r]=0$.

This general model provides the basis for simulating the different regional dynamics through varying the mean population and applying a probability function that reflects the frequency with which catastrophic environmental conditions occur; when conditions are reasonable $\alpha = -0.0252$, and when harsh, $\alpha = -2.046$ with $\beta = 0.822$.

The graph starts with the observed population at the first survey date, and show an example of a typical output.

The model effectively simulates the regional variations in mean population level and annual fluctuations that are seen in survey data with a consistent error term, suggesting ecological coherence. The model is less good at simulating the extremes of observed data with simulated crashes tending to result in lower populations than with observed data. We also note that using the selected parameters the model is incapable of delivering the highest observed survey mean population count ($1.4$ m ha$^{-1}$) from north-east Scotland. Despite these limitations, the model did provide a reasonable simulation when tested, see Figure 2, reflecting the fact that the negative feedback in the data is more likely to be a general property of $T.\ paludosa$ dynamics rather than just an occasional or regional phenomenon.

The analyses in this study show that there is strong evidence that $T.\ paludosa$ populations are regulated by density-dependent negative feedback, but we do not believe natural enemies are the cause of this relationship. Cannibalism is a more likely explanation (Blackshaw & Coll 1999).
Researchers associated a ‘crash’ in leatherjacket numbers in 1959 with a dry August/September (Milne et al. 1965). Such population crashes are apparent from the survey data but what is clear from the analysis is that the negative feedback relationship between rate of population growth and relative abundance still applies irrespective of the environmental conditions.

Over the time scales of the published surveys, the models are not predictive. Over shorter timescales, and given an awareness of general population starting levels, they will be useful in scenario modelling. In particular, it should be possible to model grass-based arable rotations because the effect of cultivations on leatherjacket numbers is known (Blackshaw 1988). This is especially relevant to organic systems where cultural control provides the only current economic option for the management of this pest.

Acknowledgements

We thank Davy McCracken and Collette Coll of the Scottish Agricultural College for supplying unpublished survey data, and the support of the Perry Foundation, South Devon Organic Producers and DEFRA through the Sustainable Arable LINK programme.

References


Challenges in using *Metarhizium anisopliae* for biocontrol of sugarbeet root maggot, *Tetanops myopaeformis*

Stefan T. Jaronski¹, Cynthia Fuller-Schaeffer¹, Kerstin Jung², Ayanava Majumdar³, Mark Boetel³

¹USDA ARS Northern Plains Agricultural Research Lab, Sidney MT; ²Federal Biological Research Center for Agriculture and Forestry, Institute for Biological Control, Darmstadt, Germany; ³Department of Entomology, North Dakota State University, Fargo ND

Abstract: *Metarhizium anisopliae* is under development as a microbial pest control agent of the Ulidiid fly, *Tetanops myopaeformis* (Sugarbeet Root Maggot), the most serious sugar beet pest in the United States. The fungus can be deployed by several means to create a “minefield” of infectious spores in the habitat of young larvae migrating to the developing root: (1) placing conidia on/in the seed coat to allow the fungus to colonize the rhizosphere; (2) applying *Metarhizium* granules around the seed at planting, much like insecticide granules; or (3) applying an aqueous spray of spores at or before peak fly oviposition to a narrow band of soil at the base of plants, allowing spores to soak into the top 1 cm of soil, where eggs are laid. A number of constraints could affect successful control by limiting *Metarhizium* survival before spores can contact larvae and influence conidial acquisition. We have examined several factors affecting *Metarhizium* performance: conidial and fungal granule concentrations in the soil; soil type (texture), moisture, and temperature influences on efficacy and persistence; the extent of rhizoplane/rhizosphere colonization; the effect of common planting-time fungicides; and interactions with a sample of rhizosphere-associated bacteria. The value of planting-time granule and preovipositional conidial sprays in high and low insect pressure situations was also determined in replicated field trials.

Introduction

The sugar beet, *Beta vulgaris* L., was introduced into the United States in the 1890s, and now encompasses some 570,000 ha across 12 states, with a total annual production of 30,624,000 tons, about half of all refined sugar produced domestically. Depending upon the locale, sugar beet is typically grown in a 2-year or 4 to 5-year rotation with cereals, legumes, alfalfa, potato and maize. The majority of sugarbeet farms are <200 ha, with about 40% <60 ha.

Perhaps the most significant insect pest of sugar beets is the sugarbeet root maggot (SBRM), *Tetanops myopaeformis* (Diptera: Ulidiidae), which is native to North America. Its original hosts were probably wild Chenopodiaceae, but as sugar beets became prevalent in the early 1900s the flies adapted. It was not until the 1950s that the fly was recognized as a pest. In the early 1970s it became a serious pest, esp. in the Red River Valley, between North Dakota and Minnesota. Today it seriously impacts approximately 278,500 ha (49% of total area). Untreated, it can cause up to 40% yield loss. The insect overwinters as a diapausing third-instar larva, 30-45 cm deep in the soil. In spring, as soils warm, the larvae break diapause, migrate to within 10 cm of the soil surface and pupate. Adults emerge shortly thereafter and migrate from the previous year’s fields to the present year’s sugar beets. Adult emergence and migration can be predicted with a degree-day model and monitored by use of orange sticky traps. The female lays her eggs in the top 1 cm of soil immediately around the emergent sugarbeet seedling, usually about 5-6 weeks after planting. The main control tools
historically have been chemicals; currently terbufos and chlorpyrifos are the principal insecticides, used at planting or to a lesser extent as a post-emergent lay-by treatment. Aldicarb and phorate are used to a much lesser extent; esfenvalerate and zeta-cypermethrin sprays are sometime used to suppress adult flies.

Microbial control of SBRM has been the focus of U.S. Department of Agriculture efforts since the mid-1990s. Since 1994 research has focused on a series of *Metarhizium anisopliae* var. *anisopliae* isolates; the latest being tested is the recently commercialized F52 (Novozymes Biologicals, Salem VA). The F52 isolate is better known in Europe as BIPESCO 5, (G. Zimmerman, personal communication).

There are several ways to deploy a fungal control agent against sugarbeet root maggot. The key with all tactics is to create an infectious “minefield” of fungal spores to intercept newly hatched maggots as they migrate to the sugarbeet root. The standard approach has been to apply granules containing *Metarhizium* conidia at planting time, much in the same manner as chemical insecticide granules. The fungal conidia might be applied as a seed coat from which the fungus could colonize the growing root system. Or, one might wait until the flies move into the sugarbeet field to oviposit, then apply an aqueous spray of fungal conidia to the bases of the beet seedlings in a band-over-row spray, using conventional equipment.

There are some overarching practical constraints in deploying *Metarhizium*, or any microbial, against SBRM, to achieve market acceptance. First, a microbial needs good commercial characteristics (commercially feasible production, practical formulation(s), and satisfactory shelf life). Second, any microbial product needs also to conform to typical agricultural practices, equipment, amounts of formulation and carrier/acre applied to the crop. Major changes in farm practice, esp. ones that would require capital outlay to employ a microbial product, will be met with user resistance. A microbial product must also be cost competitive unless it offers some significant advantages; in sugar beets an upper cost limit may be on the order of US$50 per hectare. Many U.S. farmers operate in a chemical paradigm, leading to expectations of high-degree, rapid efficacy that are possible only with chemical insecticides.

**Specific challenges in the use of *Metarhizium***

*Metarhizium* in a seed-coat application
Rhizosphere, and especially rhizoplane, colonization and subsequent sporulation by the fungus are prerequisite to the feasibility of this approach. The ubiquitous seed-coat fungicides cannot interfere with colonization and sporulation, nor can the many rhizosphere-/rhizoplane-inhabiting microorganisms. Once the previous challenges have been met, the conidia must be able to survive seed pelletization, very commonplace in this industry, although hand-mixed, ad hoc, seed coating used with *Rhizobium* might be feasible.

Using green fluorescent protein transformants, we have observed that *Metarhizium* and *Beauveria* can colonize the rhizoplane of young sugarbeet seedlings in vitro, in an agar-based system. However, in gnotobiotic media -- sterile clay soil, sterile potting mix, vermiculite + 10% Hoagland’s Solution – rhizoplane colonization was not observed, regardless of whether conidia were applied to the seed coat or added to the medium. Root colonization was also not observed with Swiss chard (*Beta vulgaris var. cicla*), bean (*Phaseolus vulgaris*), or maize (*Zea mays*) seedlings. We determined that conidial germination was almost nonexistent in root exudate of 2-leaf sugar beets, but reached about 50% after 24 hr in exudate from 4-leaf beets, cabbage and chard. In contrast, germination was >95% in oat, rye or bean root exudate, in 1% neopeptone, or in Sabouraud dextrose broth. It is clear that a seed-coat approach may not be practical. We also examined the in vitro interactions between 30 rhizosphere bacteria
and each of three isolates of *B. bassiana* and *M. anisopliae*. There were qualitative differences among the fungal species and isolates in their response to the various bacteria. A general trend appears to be greater inhibition of germination by Gram negative (G-) than Gram positive (G+) species. Hyphal growth of the fungi was generally not inhibited by any of the bacteria. More G+ bacteria were inhibited by *Metarhizium* than by *Beauveria*, and fewer G- bacteria were inhibited by either fungus. The nature of the medium strongly affected the results.

**Metarhizium as a planting-time granule**

Granules must be compatible with farm practice, i.e., be of a size and bulk density that can be properly applied by existing equipment. Agricultural requirements mandate a minimal granule size of 0.5-1.5 mm diameter, and an upper practical limit of 22 kg granules/ha in sugar beets. (Conventional insecticide granules are applied at 11-17 Kg/ha.) Thus, the GranMet®-style fermentation substrate granules (AgriFutur s.r.l, Italy), based on barley, are not practical because they are larger than a pelleted sugarbeet seed. Pulverized spent substrate, on which the fungus was grown for conidial production, is more practical, but we have observed that the pulverization and mechanical size grading processes render the granules more susceptible to colonization by soil fungi once the granules have been applied, and it is difficult to create granules of a satisfactory size range without considerable wastage.

A critical concentration of granules is needed for high efficacy. In replicated bioassays using third-instar larvae in a clay soil typical of sugarbeet culture and at optimal moisture and temperature for the fungus, 4 or more granules (corn grit granules 0.5-1 mm diameter, coated with *Metarhizium* conidia and having a bulk density of 1400 granules/gram) per gram were needed for >90% efficacy (Jaronski, unpublished data). At 4 granules/cc soil one would need 158 Kg granules/ha if applied in a 12-15 cm band over the row and incorporated to a depth of 2.5 cm – clearly impractical and uneconomic. But the critical concentration of granules can be achieved at 11 Kg/ha when granules are applied using in-furrow approaches.

Permissive temperatures and soil moisture are required for *Metarhizium* outgrowth and sporulation; the latter process is crucial with mycelial granules. Fortunately, our studies have revealed that if soils are permissive for sugarbeet seed germination, conditions are permissive for the fungus.

Mefenoxam, hymexazole, and thiram are universally used in sugarbeet seed coats to protect the young seedlings from damping off pathogens. The latter is quite toxic to *Metarhizium* in the traditional *in vitro*, agar-based assays. However, when *Metarhizium* granules are placed even < 5mm from a seed, with the full complement of fungicides, fungal outgrowth and sporulation are not inhibited on water agar or moist soil (Jaronski, unpublished data). The lag time of 6-8 weeks between planting (and *Metarhizium* granule application) and SBRM egg hatch requires good persistence of the *Metarhizium* conidia. This is achievable, particularly by a new form of mycelial granule developed by USDA (Jackson and Jaronski, unpublished).

**Metarhizium in a post-emergence spray**

The tactic here is to spray a conidial suspension into the soil around the base of the plants where the flies oviposit at the immediate onset of oviposition. Timing can be close to oviposition and egg hatch, as determined by degree day models and established surveillance methods, but there are disadvantages: (1) the conidia are restricted by carrier volume limitations to the top 5-10 mm of the soil surface, a zone exposed to extreme temperature and moisture fluctuations; and (2) the presence of neonate larvae in this zone is transient, limiting exposure to conidia. The critical concentration for high larval mortality is on the order of 1x10^6 conidia/cc of soil, a level theoretically achievable with commercially acceptable rates of fungus. However, a complicating factor is the effect of soil texture and moisture on the
infectivity of conidia, most likely mediated through pore structure. In one clay soil we observed a direct relationship between moisture (10, 15, 30% water holding capacity) and conidial infectivity; in other soils this relationship breaks down, lacking consistent correlation with the sand:silt:clay ratio. In contrast, the same relationship between moisture and infectivity holds in the case of a second clay, and in a loam, while in a clay loam, and in a sandy clay loam, increasing water content does not affect fungal infectivity. However, infectivity is adversely affected in a sandy loam. In all cases conidial viability is unaffected.

This complex relationship between soil texture, moisture, and conidial infectivity may present serious challenges to the broad application of this particular tactic in controlling SBRM. Fortunately, soil temperatures are permissive for infection and pathogenesis during the critical window of larval contact with conidia, and several years of field observations indicate that conidial persistence in the soil is sufficient to potentially affect most of the neonate larvae.

Several years of field trials evaluating at-planting applications of granules and oviposition period sprays of conidia have shown us that *Metarhizium* can reduce root damage to the same extent as terbufos in low insect pressure situations. These are the situations where chemical insecticides are perhaps not really necessary but are still being used because of grower sentiment. Against high insect pressure, however, such as occurs very frequently in northeastern North Dakota and in central Idaho, efficacy of the fungus as a stand-alone control strategy is clearly insufficient. Integration of *Metarhizium* with other IPM tools is therefore needed. Coupling the fungus with resistant sugarbeet hybrids has not yet proved successful. In one trial, yield improvement from use of *Metarhizium* with an SBRM-resistant variety was not significantly different from *Metarhizium* with a susceptible cultivar (Jaronski and Campbell, 2006). Early planting, or use of a harrow or rotary hoe during cultivation, is believed to help reduce SBRM impact. It is possible that this cultural tactic could complement the use of *Metarhizium*; however, this integrated approach still needs evaluation.

The most successful approach thus far has been the use of *Metarhizium* strains with an oat or rye cover crop (Majumdar et al., 2003, 2004, 2006). Beet farmers in the Red River Valley have used such cover crops to control spring wind erosion for many years. The cereal crop is planted just before beets, and comes up before them. The cereal – rye or oats appear to be the best – is killed off with herbicides when it is 10-15 cm tall, during the normal course of the crop season. In repeated field trials a stand-alone oat cover crop provided moderate levels of root protection against heavy insect pressure when planted at a high seeding rate (374 seeds/m²); performance of cover crops in providing root protection was inconsistent among years, however. Combining rye at the high seeding rate with *Metarhizium* (applied as a granule at planting) provided the same level of control as chemical insecticides. Root injury levels in plots that received both *Metarhizium* and an oat cover crop did not differ from the stand-alone cover crop treatments, and there was greater consistency in performance of the integrated treatments. Therefore, it appears that *Metarhizium* plus a cover crop may have a fit in sugar beets. Correct placement of post emergence *Metarhizium* sprays with a dense cover crop canopy can be a challenge, however, especially when a high seeding rate is used. Oat plants are more erect compared to rye crops; consequently, it is easier to reach the target zone when applying the fungus as a postemergence spray in an oat cover. Thus, it is important to understand crop phenology and its impact.
References


Aggregation attractants for the sugar-beet weevils *Bothynoderes punctiventris* and *Conorrhynchus mendicus* (Coleoptera, Curculionidae, Cleoninae): application opportunities

Miklós Tóth¹, Lorenzo Furlan², Giovanni Campagna³, Zoltán Imrei¹, Ivan Sivcev⁴, Ivan Tomasev⁵, István Ujváry⁶

¹Plant Protection Institute, Hungarian Academy of Science, Budapest, POB 102, H-1525, Hungary; ²Department of Agronomy, Entomology, Padova University, Agripolis, Via Romea 16, Legnaro, I-35020 Italy; ³COPROB, Minerbio, BO, Via Mora 56, I-40061 Italy; ⁴Institute for Plant Prot. & Environ., Belgrade, POB 33-79, SCG-11040 Rep. of Serbia and Crna Gora; ⁵Ministry of Agriculture and Forestry, Republic of Serbia, Phytosanitary Station for Plant Protection Subotica, Rep. of Serbia and Crna Gora; ⁶Institute of Biomolecular Chemistry, Chemical Research Center, Hungarian Academy of Science, Budapest, Pusztaszeri út 59/67, H-1025, Hungary

Abstract: Earlier we discovered that a mixture of Grandlure I, II and III-IV showed attractancy for the sugar-beet weevil [*Bothynoderes (Cleonus) punctiventris*], which is a very important pest of sugar-beet in Eastern Europe. According to our most recent studies in Italy, the same compound mixture was attractive also for the closely related weevil *Conorrhynchus (Cleonus) mendicus*, which occurs in the western and southern part of Europe and in North Africa. Studies on the activity of the single components showed that only Grandlure III/IV [(Z)- and (E)-(Δ)3,3-dimethylcyclohexylidene acetaldehyde] was responsible for activity in both *B. punctiventris* and *C. mendicus*. Pitfall traps baited with our new attractant were ca 10 times more sensitive for *C. mendicus* than traps without this compound. Our present results suggest that Grandlure III/IV may be more widespread as an attractant in the subfamily Cleoninae than thought before. This is surprising, since this compound has been described so far from the pheromones of *Anthonomus* spp. (Curculioninae) and *Pissodes* spp. (Calandrinae). From a practical point of view it is of advantage that the two most important sugar-beet weevils in Europe can now be captured and monitored with the same synthetic attractant. Mass trapping efforts during two years on *B. punctiventris* showed that traps with this attractant at a density of 10 to 30 trap/ha were capable of trapping out a greater part of the population, thus the traps show perspective also for direct population control of sugar-beet weevils.

Key words: aggregation attractant, trapping, *Bothynoderes punctiventris*, *Conorrhynchus mendicus*, Coleoptera, Chrysomelidae

Introduction

The sugar-beet weevil (*Bothynoderes punctiventris* Germar) (Coleoptera: Curculionidae) is an important pest of sugar-beet throughout the central, eastern and southeastern parts of Europe (Schegolev, 1950, Hoffmann, 1966, Manninger, 1990, Sekulic, 1997). In the areas with drier climate it represents the most destructive pest of sugar-beet causing severe losses especially during outbreak periods. In the last century *B. punctiventris* destroyed over 250,000 ha of sugar-beet fields in Serbia alone (Sekulic et al., 1997).

The southern sugar-beet weevil, (*Conorrhynchus mendicus* Germar) (Coleoptera: Curculionidae) is very similar to *B. punctiventris* in both morphology and life habits. *C.
*mendicus* is widespread in the southwestern parts of Europe and in North Africa (Hoffmann, 1966), and causes damages of similar type and magnitude to those caused by *B. punctiventris* in Eastern Europe.

Trapping tools suitable to detect and to monitor sugar-beet weevils would be of great importance for their control. At present detection and monitoring is done by plastic buckets dug in the soil into which randomly crawling beetles fall in and get caught. Other methods include soil sampling to estimate population density of overwintering insects in the soil, and visual scouting for adult beetles already coming out. Both methods are very time consuming and labor intensive. An attractant-baited trap could be more precise and easier to use than present methods, could enhance effectiveness of other control measures, and could reduce pest populations by mass trapping, at the same time fulfilling perfectly the requirements of an integrated pest management system.

We recently discovered a synthetic attractant for adult beetles of *B. punctiventris* (Tóth et al., 2002a, 2002b, 2006, Ujváry et al., 2002). In the present paper we report on the activity of this attractant for *C. mendicus*, and discuss application possibilities of traps baited with this attractant for the control of sugar-beet weevils.

**Material and methods**

Field tests were conducted at several sites in Hungary, Italy and the Republic of Serbia and Crna Gora (SCG, former Yugoslavia). Trapping tests were conducted according to internationally accepted methods for such assays. For details of single tests please refer to the Figure legends or the respective reference cited.

Synthetic Grandlure components were purchased from Bedoukian Inc. (Danbury, USA) and were >95% pure as stated by the supplier. The compounds were formulated by the usual methods in rubber dispensers (for details see for example Tóth et al., 2002b).

In most trapping tests CSALOMON® TAL modified funnel traps (Tóth et al., 2002b; produced by Plant Prot. inst., HAS, Budapest, Hungary) were used. In some field tests plastic bucket pitfall traps were used.

**Results and discussion**

**Indication of attraction**

In all of the preliminary tests performed traps baited with a mixture of synthetic Grandlure I., II and III-IV caught significantly more *B. punctiventris* than the unbaited traps, strongly suggesting attractive activity of the mixture (for details pls refer to Tóth et al., 2002c, 2005, Sekulic et al., 2004). In a test in Italy on *C. mendicus*, traps baited with the Grandlure mixture caught a mean (±SE) of 1.10±0.56 weevils, vs. a mean of 0.05±0.03 in unbaited control traps (P=0.0364 by Student *t* test; a total of 46 weevils were caught in the test) (Emilia Romagna, Italy, April 30 - June 10, 2002), suggesting that the Grandlure mixture was attractive also for *C. mendicus*.

When studying the sex of the beetles captured, for *B. punctiventris*, catches of both male and female weevils were significantly higher in baited vs. unbaited traps, suggesting that the synthetic attractant was attractive for both female and male weevils (Figure 1). Sex ratio in baited and unbaited traps was similar, suggesting that attraction was equally strong for the two sexes, and thus sex ratio of captures in an attractant baited trap would represent the natural sex ratio of the population in the field. For *C. mendicus*, out of 17 randomly selected weevils from the catch with attractant baited traps, 9 were females and 8 males (L. Furlan, pers. comm.), suggesting that in this species also both sexes were attracted.
Relative importance of Grandlure components

When the relative importance of the Grandlure components was studied, traps with baits containing Grandlure III-IV (alone or in binary or ternary combinations) caught significantly more *B. punctiventris* than traps with Grandlure I, II, or their binary mixture (Figure 2), suggesting that from the three components only Grandlure III-IV [(Z)- and (E)-(Δ)3,3-dimethylcyclohexylidene acetaldehyde] was responsible for activity. Similar results were obtained in *C. mendicus* (results of these tests will be published in detail elsewhere).

Figure 1. Sex ratio of sugar-beet weevils *B. punctiventris* in traps with or without the synthetic attractant in field tests in Serbia. Test A: Zarkovci, April 5 - May 25, 1999; Test B: Pancevo, April 8 - June 20, 2000. P values by Student *t* test. Data from Tóth et al., 2002c, 2005.

Figure 2. Catches of *B. punctiventris* weevils in traps baited with Grandlure I, II and III-IV on their own or in mixtures. Columns show mean/trap/inspection + S.E. Inspections were done twice weekly. Columns with same letter are not different at P<0.05 by ANOVA, Games Howell. A total of 1025 beetles were caught in the test. (Data from Tóth et al., 2006)
Dosage tests
In subsequent dosage tests with Grandlure III-IV alone, it appeared that in *B. punctiventris* the optimal dosage may be between 300 and 3000 μg (Figure 3). In *C. mendicus*, very similarly, optimal dose range was found to be between 500 and 5000 μg (Figure 3).

**Figure 3.** Mean catches of sugar-beet weevils *B. punctiventris* or *C. mendicus* in traps baited with different doses of Grandlure III-IV. Columns with same letter within one diagram are not different at P<0.05 by ANOVA, Games Howell.

Suitability for detection and monitoring
In testing the suitability of attractant baited traps for detection and monitoring, parallel tests on *B. punctiventris* or *C. mendicus* were run at sites with extremely low population density in Hungary and Italy, respectively. Traps baited with the attractant proved to be ca. one magnitude more sensitive, catching significantly more weevils in both species than the unbaited control traps (which until now consisted the conventional trapping method used) (Figure 4). Due to the larger numbers captured, monitoring the changes in the population could also be better followed with attractant-baited traps (Figure 4).

Traps baited with the attractant performed well also in more detailed monitoring studies on *B. punctiventris*. The baited traps detected ca. one week earlier the first occurrence of the sugar-beet weevils, than conventional visual sampling (Figure. 5) (Sivcev et al., 2005). The flight dynamics was followed also more reliably with the traps baited with the attractant. Visual sampling is highly weather-dependent and fairly labor intensive. Traps with the new attractant bait show promise in both detection of immigrating beetles from overwintering sites to the new crop and in monitoring population changes throughout the season.
Figure 4. Suitability of traps baited with the synthetic attractant for detection and monitoring of the sugar-beet weevils *B. punctiventris* or *C. mendicus*.

Figure 5. Suitability of traps baited with the synthetic attractant for monitoring and detection of the sugar-beet weevil *B. punctiventris*. Pancevo, SCG, 2000. Data from Sivcev et al, 2005.

**Suitability for mass trapping**

In preliminary trials aimed at studying the applicability of attractant-baited traps for mass trapping, already the density of 10 traps/ha was capable of trapping out a sizeable proportion of the *B. punctiventris* population, while 30 traps/ha removed virtually all weevils (Tomasev et al., 2005). Farmers who owned the sugar-beet fields where the tests were set up were very
satisfied with the control of the weevil at the experimental plots (Ivan Sivcev, pers. comm.). The above results show perspective in the application of attractant-baited traps not only for monitoring and detection, but also for direct control of sugar-beet weevils through mass trapping. As yet no similar studies have been conducted in _C. mendicus._

**Conclusions**

Our present results suggest that Grandlure III/IV may be more widespread in the chemical communication of the curculionid subfamily Cleoninae than thought before. This is surprising, since this compound has been described so far from the pheromones of _Anthonomus grandis_ Boheman (Tumlinson et al., 1969, 1971), _A. eugenii_ Cano (Eller et al., 1994), _A. rubi_ Herbst (Innocenzi et al., 2001), _Curculio caryae_ Horn (Hedin et al., 1997), and also from _Pissodes_ spp. (Phillips et al., 1984, Booth et al., 1983). The above mentioned weevils belong to the subfamilies Curculioninae and Calandrinae, while the sugar-beet weevils belong to Cleoninae.

In the course of our experiments _C. mendicus_ responded to the attractant very similarly to _B. punctiventris_ in all aspects studied. From the practical point of view it is of great advantage that both important European sugar-beet weevils, _B. punctiventris_ and _C. mendicus_ can be attracted to traps with the same synthetic aggregation attractant.

**Acknowledgements**

This research was partially supported by grant NKFP 4/012/2004 OM of the Hungarian Ministry of Education.

**References**


Metarhizium spp. against locusts and grasshoppers – a short review and future prospects

Barbara Pernfuss¹, Roberto Kron Morelli², Roland Zelger³, Hermann Strasser¹
¹ Institute of Microbiology, Leopold-Franzens-University, Technikerstrasse 25, A-6020 Innsbruck, Austria; ² Agrifutur s.r.l., Via Campagnole 8, I-25020 Alfianello (Brescia), Italy. ³ Land- und Forstwirtschaft-liches Versuchszentrum Laimburg, Laimburg 6, I-39051 Pfatten/Auer, Italy.

Abstract: A short review on the use of Metarhizium anisopliae var. acridum and M. anisopliae var. anisopliae as biocontrol agents (BCAs) against locusts and grasshoppers is presented. Mainly two projects, i.e. i) LUBILOSA (LUtte Biologique contre les LOcustes et SAutériaux) in Africa, and ii) CSIRO in Australia aimed to study the efficacy of BCAs against locust and grasshopper pests and developed products on the basis of Metarhizium spp., which were efficacious in the field and are accepted by the farmers. Currently, a plague of Siberian Grasshoppers (Aeropus sibiricus) devastates ecologically sensible alpine regions. Furthermore, mainly from South European Countries augmented reports are arriving which concern increasing crop ruination because of grasshoppers and locusts which accelerate possibly because of the global warming. This is why both the knowledge of the above mentioned projects and our own experience with biological control and Metarhizium will be tied in order to develop an adequate application of Metarhizium spp. for the control of grasshopper and locust pests in Europe.

Key words: Metarhizium anisopliae var. acridum, Metarhizium anisopliae var. anisopliae, grasshoppers, locusts, Aeropus sibiricus, biocontrol, biocontrol agents

Introduction

Unique landscapes and traditional crops are under increasing threat from a number of insect pests that have proven particularly difficult to control. At the same time concerns over the deleterious effects of chemical insecticides on human and environmental safety have provoked potent impulses for the processing of microbial control agents for use in biological and integrated control.

Amongst the most promising counteragents found and advanced to control insect pests are Metarhizium species. Pests which are (tried to be) controlled with various customised products made of Metarhizium spp. include the larvae of Scarabaeidae and Curculionidae (e.g. Amphimallon solstitialis, Phyllopertha horticola, Otiorrhynchus sulcatus, Bothynoderes punctiventris), Elateridae (e.g. Agriotes obscurus), the grape phylloxera Daktulosphaira vitifoliae and the new exotic pest Diabrotica virgifera virgifera. Worldwide specific strains of Metarhizium spp. are also being used to control grasshoppers, termites, thrips, and its use in the control of malaria-transmitting mosquitos is under investigation.

Research on Metarhizium against locusts and grasshoppers began in the early 1990s in both Africa and Australia. Locusts are amongst the most dramatic and devastating of the insect pests. Locust populations can cover more than 1000 km² and cause immense damage as they swarm across the countryside. Locust control is therefore the subject of intense political interest, especially during outbreaks and both national authorities and donor communities...
have been keen to develop control methods that are less insecticide dependent and more environmentally friendly. Presently it seems that entomopathogenic control could provide a very effective solution. The long-running project, LUBILOSA (LUtte Biologique contre les LOCustes et SAutériaux), has recently seen its efforts crowned with success with the launching of two commercial products based on the entomopathogenic fungus *Metarhizium anisopliae* var. *flavoviride* for the control of grasshoppers and locusts (Lomer et al., 1999; Milner, 2000; Lubilosa, 2006).

In Australia preliminary results were so promising that a joint project involving the Australian Plague Locust Commission, CSIRO Entomology in Queensland, NSW Department of Primary Industries, the Wingless Grasshopper Group and a commercial partner begun in 1997. Between 1997 and 2000 field trials covering areas of several hundred hectares showed that *Metarhizium* could reduce the numbers of locust hoppers by > 90 %. The fungus kills the insect with the greatest mortality occurring sometime between seven to 15 days after treatment (depending upon ambient temperature). This success led to the first operational use of *Metarhizium* anywhere in the world during 2000 – 2001 in which over 20 000 hectares of bands and swarms of Australian plague locust were treated (Taylor, 1998; Hunter et al., 1999; Hamilton et al., 2000; Kooyman, 2003; Csiro Australia, 2006).

Currently, possibly provoked by exceptional hot and dry summers, a plague of Siberian Grasshoppers (*Aeropus sibiricus*) devastates ecologically sensitive alpine regions in South Tyrol (Italy). This species only occurs on dry alpine grassland or in the undergrowth of alpine roses (*Rhododendron spp.*) between an altitude of 1 000 and 2 600 meters.

A possible application of *Metarhizium* spp. for the control of *Aeropus sibiricus* in South Tyrol will be presented.

**Material and methods**

In the finished EU RTD-project BIPESCO (FAIR6-CT98-4105) two *Metarhizium* strains have been identified as highly pathogenic in laboratory and preliminary field trials, mainly against soil dwelling insect pests. One of the strains – BIPESCO 5 – turned out to be notably adequate for the production of commercial products, as the conidia were rather unsusceptible to the sometimes rigid conditions of mass production (i.e. drying) and kept high viability and germination rate.

**Origin and natural occurrence**

*Metarhizium anisopliae* BIPESCO 5 was collected on *Cydia pomonella* in Austria and isolated in Darmstadt by Gisbert Zimmerman of BBA (Biologische Bundesanstalt für Land- und Forstwirtschaft, Darmstadt, Germany). It is wild-type, and it has not been deliberately mutated. BIPESCO 5 is characteristic and typical of the variety *Metarhizium anisopliae* var. *anisopliae*, which has a world-wide distribution.

**Intended use**

GRANMET® (*M. anisopliae* BIPESCO 5) the commercial product, is intended for the control of insect pests. In particular, the product targets are soil-dwelling weevil (Coleoptera: Curculionidae) pests including: *Strophosoma melanogramma*, *Strophosoma capitatum*, *Otiorhynchus* spp. and the soil dwelling beetles (Coleoptera, Melolonthidae) including: *Phyllopertha horticola*, *Amphimallon solstitialis*. It is intended for the use in open fields, particularly in forestry, nurseries and in grassland and meadows, viticulture (vine), alpine meadows, ornamentals, Christmas tree and decorative Christmas greenery plantations (i.e. *Abies nordmanniana* and *Abies procera*).
The product GRANMET®, which can be applied by means of slit seeding (granules), drenching soil (lance-injector) or by spraying (wettable conidia powder) will be tested to control Siberian Grasshoppers in the alps within the next summer season.

Results and discussion

Location
A plague of Siberian Grasshoppers (*Aeropus sibiricus*) last summer has devastated ecologically sensitive alpine regions in South Tyrol (Italy). The valley “Ultental” near to Meran is an original place where mountain farmers operate their farmyards. In this region the highest alps occur in Europe. Up to an altitude of more than 2 000 meters livestock is grazed and herbal meadows are cut.

![Map of South Tirol (Italy) showing the Ultental valley.](image)

Figure 1. Map of South Tirol (Italy). The valley “Ultental” spans from Meran to the Southwest.

Inspection
In summer 2006 alpine meadows in “Ultental” were inspected. At an altitude of 2 000 m the meadows were found bare of grass and herbs. Up to 1500 individual of *Aeropus sibiricus* per squaremeter were counted.

Critical issues to be solved
For an application of our Product GRANMET® to control locusts and grasshoppers in Europe, it will be necessary to make sure first, that *M. anisopliae* BIPESCO 5 is virulent against these pests.

The life cycle of the pest organism – in this case of the Siberian grasshopper *Aeropus sibiricus* - has to be studied in detail in order to decide if it is more effective to control adults and/or nymphs and/or eggs. From this decision it will depend how the active substance (*M. anisopliae*) will be applied. If spraying of GRANMET® is more effective and thus preferred, a suiting UV-protection and eventually a special spraying equipment will be necessary for the conidia of *M. anisopliae* BIPESCO 5.
These and other key issues (i.e. effective dose) will be investigated next season by field testings of our product against the Siberian grasshopper *Aeropus sibiricus* in the alps of South Tirol in “Ultental”.

Figure 2. Grasshopper plague (*Aeropus sibiricus*) in “Ultental” (http://www.ueliraz.ch/Wallis/heuschrecke.htm)

References


Lubilosa 2006: http://www.lubilosa.org/exsumm.htm (last access: 20.11.2006; MEZ 14:00)


Taylor, R. 1998: Bugs beware – Here comes the good fungus. An oil based formulation containing the *Metarhizium* fungus has been developed to control plague locusts. ECOS 95, April – June 1998, 10.1071_ISSN0311-4546EC95p5.

http://www.ueliraz.ch/Wallis/heuschrecke.htm (last access: 20.11.2006; MEZ 15:00).
Persistence of GRANMET®, a *Metarhizium anisopliae* based product, in grape phylloxera-infested vineyards

Martin Kirchmair¹, Marc Hoffmann², Sigrid Neuhauser¹, Hermann Strasser¹, Lars Huber³
¹Leopold Franzens-University of Innsbruck, Technikerstr. 25, A-6020 Innsbruck, Austria; ²Johannes Gutenberg-University of Mainz, Institute of Zoology, D-55099 Mainz, Germany
E-mail: martin.kirchmair@uibk.ac.at

Abstract: Grape phylloxera (*Daktulosphaira vitifoliae* Fitch) is one of the most serious grape pests worldwide. After successful bio-tests and pot experiments with the entomopathogenic fungus *Metarhizium anisopliae* against grape phylloxera a field trial was started in spring 2003 in the German Rheingau to assess the efficacy of *M. anisopliae* against this aphid. In 2004 a second phylloxera infested vineyard was treated with GRANMET, Agrifutur s.r.l.. Three months after application an increase of the *Metarhizium* density in soil could be observed in both vineyards (>5 x 10^3 cfu g^-1 dry wt soil). Compared with untreated plots a lower grape phylloxera-infestation could be observed in the *M. anisopliae*-treated plots. One year after treatment the maximum density of *Metarhizium* was estimated (1 x 10^4 cfu g^-1 dry wt soil), accompanied with very low grape phylloxera infestations. Two years after treatment a control effect on grape phylloxera could still be observed whereas the density of the BCA in soil decreased. Three years after treatment no effect on the grape pest was detectable and the *Metarhizium* density in soil had decreased to a value similar to that in the control plots (<3 x 10^3 cfu g^-1 dry wt soil). Therefore, a periodically application is necessary for an efficient control of grape phylloxera.

Key words: viticulture, entomopathogen, Hyphomycetes, *Daktulosphaira*, control

Introduction

Grape phylloxera, *Daktulosphaira vitifoliae* (Fitch), is a serious pest of commercial grapevines worldwide. On fresh roots of vine, the grape phylloxera causes so called nodosities, beak-like swellings, as a result of feeding activity. High populations of this pest can result in premature defoliation, reduced shoot growth, reduced yield, and reduced quality of the crop, and even crop death. Although, the use of systemic insecticides as well as fungal biocontrol agents are in discussion, currently no registered chemical or biological control agents against phylloxera are available in Europe.

The efficacy of strains of *Metarhizium* against grape phylloxera was demonstrated by pot experiments (Kirchmair et al. 2004a). As a direct observation of infected pests is very unlikely – grape phylloxera is less than 1 mm in size and dead individuals are rapidly mineralised – we resort to the assessment classes for phylloxerya infestation published by Porten and Huber (2003). Treated plants could be ranked in lower assessment classes as untreated plants. The *Metarhizium* density was higher in treated pots with phylloxera infested plants than in those without phylloxera infestations. In 2003 a first randomised block designed field trial was established. A few months after treatment an effect on grape phylloxera populations was detectable (Kirchmair et al. 2004b). In this paper the fate of *M. anisopliae* in phylloxera infested vineyards is discussed.
Material and methods

Field trial layouts
A full randomised block design (EPPO, 2004) was used for the *M. anisopliae* risk assessment trial in a vineyard near Geisenheim, Hessen, Germany. Untreated plots and plots treated with sterile barley kernels (33 kg ha\(^{-1}\)) served as controls. *M. anisopliae* colonised barley (50 kg ha\(^{-1}\)) was one-time applied by a sowing machine in combination with a rotary harrow in May 2003. A second trial site was established in May 2004.

Quantification of fungal BCAs in the soil
Soil samples (depth 0-10 cm and 10-20 cm) were taken with a core borer. Samples from each layer were mixed, air-dried, and sieved through a 2 mm sieve. Ten gram sub-samples from each depth (three replicates) were added to 40 mL 0.1 % (w/v) Tween80®, shaken at 150 rpm for 30 min, and then treated in an ultrasonic bath for 30 s. Agar plates selective for *Metarhizium* (Strasser et al. 1996), supplemented with 22 g L\(^{-1}\) glucose monohydrate were inoculated with 50 µL of these soil suspensions or dilutions thereof (four replicates per sub sample) and were then incubated for 14 days at 25 °C and 60 % relative humidity (RH). Colonies formed by *Metarhizium*, are given as CFU g\(^{-1}\) soil dry weight.

Results and discussion

Metarhizium density
Roots of grape vine are distributed very in-homogenous. Most roots can be found underneath the row and much less in the tramline. Application underneath the row would therefore guarantee the contact of the BCA with grape phylloxera. As this form of application is not manageable with the agricultural machinery routinely in use by winegrowers, we decided in favour of a tramline-only application. No relevant density of *M. anisopliae* could be found in the soil before application. In both trial sites, the density in the tramline of treated plots was sufficiently high after application (10^5-10^6 CFU g\(^{-1}\) dry soil), while according to the application technique only low densities were detected underneath the row of treated plots (≤10^3 CFU g\(^{-1}\) dry soil; Fig. 1). One year after the treatment an increase of *M. anisopliae* density could be observed underneath the row in treated plots. In trial site 1 a decrease was observable two years after application, while in trial site 2 the *Metarhizium* density decreased in the third year after application (Figs. 2-3, Table 1). We interpret these findings that *Metarhizium* spores or other viable propagules are carried by vectors (e.g. collemboles) from the tramline underneath the row where a host for *Metharizium* – grape phylloxera- is available. A higher density of *Metarhizium* should therefore be suspected in soil close the roots and rootgalls than in soil distant from the roots. To proof this belief, we divided the soil sample from underneath the row of treated plots into two sub-samples (“nearby the roots” – “aloof from the roots”). At both trial sites we found a distinct higher *Metharizium* density in soil close to the roots (trial site 1: 7.8×10^3 ± 1.5 × 10^3 versus 5.7×10^2 ± 3.6×10^2; Trial site 2: 1.2×10^3 ± 3.0×10^2 versus <1×10^2; Fig. 4).
Table 1. *Metarhizium* density in the upper 10 cm of the vineyard soil [CFU g⁻¹ dry weight of soil] given in the form “median ([Q3-Q1]/2)”; Trial site 1.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>&lt;1×10⁴</td>
<td>&lt;1×10⁵</td>
<td>&lt;1×10⁵</td>
<td>2.8×10⁶</td>
<td>&lt;1×10⁷</td>
<td>&lt;1×10⁷</td>
<td>&lt;1×10⁷</td>
</tr>
<tr>
<td><strong>GRANMET: tramline</strong></td>
<td>&lt;1×10⁴</td>
<td>6.7×10⁴</td>
<td>2.1×10⁴</td>
<td>5.4×10⁴</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td><strong>GRANMET: row</strong></td>
<td>&lt;1×10³</td>
<td>4.2×10³</td>
<td>2.1×10³</td>
<td>8.2×10³</td>
<td>3.4×10⁴</td>
<td>2.7×10³</td>
<td>4.8×10³</td>
</tr>
</tbody>
</table>

Table 2. *Metarhizium* density in the upper 10 cm of the vineyard soil [CFU g⁻¹ dry weight of soil] given in the form “median ([Q3-Q1]/2)”; Trial site 2.

<table>
<thead>
<tr>
<th></th>
<th>Before application</th>
<th>June 2004</th>
<th>June 2005</th>
<th>June 2006</th>
<th>August 2006</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>&lt;1×10⁴</td>
<td>1.2×10⁵</td>
<td>&lt;1×10⁵</td>
<td>1.2×10⁵</td>
<td>&lt;1×10⁷</td>
</tr>
<tr>
<td><strong>GRANMET: tramline</strong></td>
<td>&lt;1×10⁴</td>
<td>3.9×10⁵</td>
<td>n.d.</td>
<td>8.5×10³</td>
<td>n.d.</td>
</tr>
<tr>
<td><strong>GRANMET: row</strong></td>
<td>&lt;1×10³</td>
<td>1.2×10⁵</td>
<td>7.3×10³</td>
<td>3.5×10⁴</td>
<td>4.9×10³</td>
</tr>
</tbody>
</table>

Figure 1. Development of *Metarhizium*-density in a phylloxera infested vineyard (trial site 1) after tramline application with GRANMET. Density is given in CFU g⁻¹ dry-weight of soil.
Figure 2. Development of *Metarhizium*-density underneath the row in a phylloxera infested vineyard (trial site 1) after tramline application with GRANMET. Density is given in CFU g$^{-1}$ dry-weight of soil.

Figure 3. Developing of *Metarhizium*-density underneath the row in a phylloxera infested vineyard (trial site 2) after tramline application with GRANMET. Density is given in CFU g$^{-1}$ dry-weight of soil.
Figure. 4. *Metarhizium*-density underneath the row (trial site 1): A distinct higher density is detectable from the sub-sample “nearby roots”.

**Efficacy of the GRANMET® treatment**

In *Metarhizium* treated plots a reduction of phylloxera infestation could be estimated (Kirchmair et al. 2004b). Two month after treatment the infestation frequency by grape phylloxera was lower in the tramline of *Metarhizium* treated plots. Also the infestation intensity could be ranked within lower assessment classes in the tramline of treated plots. An effect of *Metarhizium* was observable also one year and, but not as clear, two years after application. In accordance with the *M. anisopliae* density no effect was detectable three years after treatment.

**Conclusion and outlook**

Trial site experiments with GRANMET suggest that *M. anisopliae* can be use to control grape phylloxera. Although roots are mainly found underneath the row, a tramline-only application of *Metarhizium* is goal-orientated. After one to two years *M. anisopliae* can be detected underneath the row. No effect was observable three years after treatment. A periodically application is necessary for an efficient control of grape phylloxera. The exact period remains to be tested. For a final assessment of the efficacy of GRANMET, field trials in different vineyard soils as well as climatic influences have to be taken into consideration.

Future work will be required to estimate how and in which amount *M. anisopliae* is spread by different vectors underneath the row. It is one task of further investigations to track the development of the *Metarhizium* density underneath the row in periodically GRANMET treated vineyards.

**Acknowledgements**

The authors wish to thank R. Pöder, M. Porten, G. Eisenbeis and E.H. Rühl for helpful discussions. This work was supported by the Forschungsring des Deutschen Weinbaus, the Bundesanstalt für Landwirtschaft und Ernährung, the Hessian Ministry of Agriculture, the Feldbausch Foundation, Department of Biology, University of Mainz and the Heinrich-Birk-Gesellschaft.
References

Are genetic algorithms a “magic bullet” for optimising cultivation conditions for entomopathogenic fungi?

Stefan Hutwimmer, Wolfgang Burgstaller, Hermann Strasser
Institute of Microbiology, Leopold-Franzens University Innsbruck, Technikerstraße 25, 6020 Innsbruck, Austria

Abstract: In this article, basic considerations about the design of cultivation media for entomopathogenic fungi, its importance and its barriers are depicted. Genetic algorithms are proposed to improve this situation and their fundamentals and advantages are presented.

Key words: fermentation, nutrient medium, stochastic search strategy, insect-pathogenic fungi.

Introduction

Nutrition influences the morphology and metabolic state of organisms, and thereby their development. For entomopathogenic fungi it is well known that cultivation conditions strongly affect mycelium production, spore yield, and the expression of virulence parameters. In the case of *Metarhizium anisopliae*, nutritional conditions were shown to have strong impact on spore endogenous reserves (Hallsworth & Magan 1994, 1995) and surface carbohydrates (Ibrahim *et al.* 2002), which contribute to spore longevity, germination, and adhesion properties. Nutrition further influences the expression of cuticle-degrading enzymes like lipases, chitinases or proteases (e.g. Shah *et al.* 2005): the intensively studied protease pr1 is induced by a proteinaceous compound of insect cuticle or de-repressed under starvation conditions, and repressed under excess nutrients (St. Leger *et al.* 1988, Paterson *et al.* 1994a, b). Because such nutrition and cultivation dependent factors contribute to the ecological fitness of entomopathogenic fungi, their stimulation and induction during production should not be neglected.

Barriers to medium design

Designing growth media with enhanced performance for fungal biopesticides is crucial because a given medium may be ideal for mass production of conidia but these conidia must not necessarily exhibit the demanded virulence. Other desired properties of conidia are resistance to desiccation, high germination ability, and overall biocontrol efficacy. Thus for medium optimisation, the simultaneous control of several essential “fitness parameters” is of importance. Classical, trial-and-error based approaches of medium design can fail not at least for combinatorial reasons. The number of experiments requested is unfeasible: considering only 16 medium components with three concentrations each, the possible combinations to be tested already exceed a number of 43 millions. This situation is getting even more discouraging if more than one target factor (e.g. conidial yield, germination speed, and protease production) is to be evaluated and optimised; data capture and data mining are critical. As Kennedy and Krouse (1999) recapitulated, medium design is a laborious, expensive, open-ended, and often time-consuming process. To improve this situation, we
propose to use a genetic algorithm as a guided search strategy for the design of optimised production media for fungal biocontrol agents.

Fundamentals of genetic algorithms

Genetic algorithms, introduced by John Holland in the 1960s, are guided random search methods and categorised to ‘Evolutionary algorithms’/‘Heuristic optimisation techniques’. The fundamental principle of genetic algorithms is the simulation of the mechanisms of evolution of organisms: ‘selection’, ‘mutation’ and ‘recombination’ (cross-over). The application of genetic algorithms for medium design is a clear recent trend (Kennedy & Krouse 1999) and claims some impressive results: Recent medium optimisations focusing on different target parameters (Weuster-Botz et al. 1995, Marteijn et al. 2003, Bapat & Wangikar 2004, Franco-Lara & Weuster-Botz 2005, Engelking et al. 2006) showed process improvements of up to 600 % following a very low number of experiments (20 parallel experiments and five experimental runs optimising nine medium components and five concentration levels each).

Unlike classical statistical designs, stochastic search strategies are generally based on the following fundamental procedure: “(i) generate a first parallel experimental approach, (ii) determine the values of the target function, (iii) generate new experimental points (‘next generation’) in the vicinity of the best experimental points of the previous generation by correlating the number of new experimental points with the quality of the previous experimental points relative to the target function (fitness) and by fitting the step width around a previous experimental point to the global optimisation success, and (iv) repeat steps two and three until the truncation criterion is fulfilled” (Weuster-Botz 2000). Consequently, the weakness of classical statistical designs can be bypassed: the time-consuming and often unfeasible identification of ‘important’ medium components and the generally necessary assumption of uni-modality of the response surface are not necessary.

Genetic algorithms alter medium compositions by ‘selection’, ‘recombination’, and ‘mutation’ fundamental to evolutionary processes. Randomly generated ‘individuals’ (media) representing ‘population 0’ will be evaluated for their fitness: the growth and development (conidial yield, protease expression, or other key factors) of the fungal production strain on the respective nutrition media will be monitored and assessed. Based on these fitness values, best media will be selected for recombinational and mutational transformations. They are allowed to ‘mate’, thereby recombining their ‘genetic information’ (medium composition) and generating new individuals. These new individuals are additionally altered by mutation, which here are infrequent (normally a mutation frequency of 5 %) and slight modifications (for example the change of a trace element concentration). After the fitness evaluation of these new individuals, a replacement scheme biased towards fitter individuals merges the new individuals (children) and the parent individuals for the next generation (Figure 1). Out of many varying replacement schemes, ‘generational replacement’ is the simplest: the current population is completely replaced by the offspring. Alternatively, in elitism, all children and the n-best elements of the parent generation (the elite) will be passed on to the next generation (population 1). Selected media (individuals of the new population) undergo the same sequence of transformation (recombination and mutation), evaluation and replacement. Hence, after a number of such highly directed searches, it is possible to end up close to the global optimum.

In silico, it is recommended to develop individual encodings for respective optimisation problems. Special operators, which regulate recombinational and mutational transformations, are necessary to maintain constraints pre-defined by the microbiological experimentator. Such
constraints can be set due to limits of the organism (e.g. no growth above a given osmolality),
knowledge about the organism’s metabolism (e.g. enzyme de-repression at starvation
conditions), or economical considerations.

Latest software developments (e.g. Wagner & Affenzeller 2005) enable the usage of
improved algorithms (Affenzeller & Wagner 2004, 2005) or multi-objective experimental
optimisation (Link & Weuster-Botz 2006) using Pareto optimality. Pareto optimality, in short
meaning that a member of the efficient set is not dominated by any other, enables the search
for preferred solutions in multi-objective optimisations. Preferred solutions are subjectively
managed by the decider.

Figure 1. Fundamentals of genetic algorithms in medium design. Starting from a random set
of cultivation media, best media in respect to the key factors are selected and transformed
using the principles of evolution. Following the replacement scheme elitism, all children and
best parents will then be merged to form the next population, whose members itself undergo
events of selection and transformation.
Conclusions

Genetic algorithms are gaining importance in biotechnological optimisations because user-friendly computer programs become available (Kennedy & Krouse 1999). Main advantages of computer-aided genetic algorithms are that they can handle a large number of medium components and target variables. The direction for further medium development is automatically set and no new guessing is necessary at each round of experiments.

Genetic algorithms, thus, are proposed to become more and more useful for the optimisation of cultivation conditions for entomopathogenic fungi, as well.

Acknowledgements

The many helpful discussions with Reinhold Pöder, University of Innsbruck (Austria), and Stefan Wagner, University of Applied Sciences, Hagenberg (Austria), are gratefully acknowledged.

References

Paterson, I. C., Charnley, A. K., Cooper, R. M. & Clarkson, J. M. 1994a: Specific induction of a


Assessment of virulence test-systems for quality assurance using sub-cultivated *Beauveria brongniartii* conidia

Angelika Loesch, Stefan Hutwimmer, Barbara Pernfuss, Hermann Strasser
Institute of Microbiology, Leopold-Franzens-University Innsbruck, Technikerstrasse 25, 6020 Innsbruck, Austria

Abstract: Producers of biocontrol agents must describe and specify assay methods for product standardisation to ensure its purity and uniformity. Based on the approved and registered *Beauveria brongniartii* isolate BIPESCO 2, conidia from three different batches ((i) stored in liquid nitrogen; (ii) isolated from a mycosed *Melolontha melolontha* larva; (iii) harvested from MELOCONT®-Pilzgerste) were sub-cultivated up to ten times on four types of nutrient media. The harvested conidia were tested for their virulence against *M. melolontha* and *Tenebrio molitor* larvae using the BIPESCO standard operation method for bioassays. Conidia were also characterised for their carbon utilisation profile using the BIOLOG™ SF-P2 and BIOLOG™ SF-N2 microtiter plates (128 carbon sources in total). The utilisation rates of carbohydrate compounds and -groups, respectively, were correlated to the virulence behaviour of sub-cultivated conidia with the aim to identify virulence markers. Furthermore, the radial growth of fungal colonies and their conidiation was assessed after each transfer. These assays confirmed that a successive transfer of *B. brongniartii* conidia in vitro is possible at least for five times without a demonstrable loss of virulence. The most relevant method for virulence control of *B. brongniartii* will be presented, which is a main issue for quality assurance of microbial pest control agents.

Key words: *Beauveria brongniartii* BIPESCO 2, quality assurance, virulence, sub-cultivation, attenuation, bioassay, carbon utilisation profile, BIOLOG™

Introduction

Producers of biocontrol agents (BCAs) are responsible for quality assurance of their products. High quality of a product is given by its efficacy (in case of entomopathogenous fungi this requests the utilisation of highly virulent strains), its purity (contaminations must be prevented as far as possible), its stability (production strains should not mutate rapidly, so that the maintenance of the cultures and the storage of the product over a longer period of time is possible), and its persistence in the environment after application (Butt 2002). Producers of biocontrol agents have to describe assay methods, preferably as standard operation protocols, to ensure these key issues of quality (European Commission 2003). It is important for producers that assay methods are simple, inexpensive and time-saving.

Virulence is the ability of an entomopathogenous fungus to cause death. The virulence of a fungal strain is not defined by a single trait, but by the combination of several factors, such as the germination rate and the germination speed of conidia, or the biochemical components of the invasion process (the expression of enzymes like proteases, chitinases, lipases, or esterases). Two key attributes of hypervirulent isolates are their ability to kill in a relatively short period of time (i.e. low LT$_{50}$ value) and to cause high mortality at relatively low doses (i.e. low LD$_{50}$ value; Butt 2002).
Variability in fungal morphology and physiology after repeated sub-culturing on artificial media is a well-known phenomenon and, in the case of pathogenic fungi, may result in altered (usually reduced) virulence levels (Pernfuss et al. 2004). Various terms have been used to describe this phenomenon, commonly it is termed attenuation. Attenuation of virulence has been observed in nearly all major taxa of entomogenous fungi, but strains differ in the rate at which they decline in virulence (Butt et al. 2006). Attenuation can be a problem for storage of production strains and for the production of BCAs, because prior to and during the commercial production process several passages of the entomopathogens without any contact to the target hosts are common. This is why a series of studies deals with the maintenance and storage of fungal isolates and the effect of different media and conditions on the attenuation of virulence (Pernfuss et al. 2004).

The aim of this work was (i) to observe virulence behavior (attenuation) of B. brongniartii conidia during sub-cultivation on artificial media and to confirm high virulence of stored conidia and of the commercialised product (quality assurance), respectively, (ii) to find correlations between morphology, conidiation, carbon utilisation and virulence in order to develope methods which might be used to determine virulence of fungal strains faster and simpler, and (iii) to check new nutrient media, that might prevent loss of virulence during sub-cultivation.

Materials and Methods

**Fungal strain**

The *Beauveria brongniartii* strain BIPESCO 2 (IMBST 95041), which is the production strain for MELOCONT®, was used in this study. The strain was isolated from *M. melolontha* infested soil in Kramsach, Tyrol, Austria by Strasser in 1995. Three different batches of BIPESCO 2 conidia were used for the experiments: conidia stored as spore-skimmed milk suspension in liquid nitrogen for 15 months, conidia isolated from a mycosed *M. melolontha* larva and conidia directly harvested from the commercialised product MELOCONT®-Pilzgerste (batch from January 2006).

**Sub-cultivation**

These three batches of conidia were sub-cultivated up to ten times on four types of nutrient media in intervals of 14 days by using pure conidia suspensions: Conidia were removed from agar plates with a sterile needle and diluted in 10 mL of 0.1 % (w/v) Tween 80. The suspensions were sucked through a cotton plug of a sterile pipette (used upside-down) to remove mycelial fragments (Newmeyer 1990). Conidia suspensions were plated diluted and undiluted on agar media to get (i) single colonies for morphological characterisation and (ii) dense mycelium/high amount of conidia for the other experiments. Incubation conditions were set to 25 °C and > 65 % r.h.

**Nutrient Media**

The following four types of nutrient media were used for the sub-cultivations (units L⁻¹): (i) Sabouraud-2-Glucose agar (S2G), the standard medium to cultivate *Beauveria* spp.: 30 g Sabouraud-2%-Glucose-Bouillon, 15 g agar (Laengle et al. 2005); (ii) complete agar medium (CAM; Tarocco et al. 2005): 0.4 g KH₂PO₄, 1.4 g Na₂HPO₄*2H₂O, 0.6 g MgSO₄*7H₂O, 1 g KCl, 0.7 g NH₄NO₃, 10 g glucose-monohydrate, 5 g yeast extract and 15 g agar; (iii) modified complete agar medium (CAMm) as a synthetic medium: CAM without yeast extract but amended with trace elements and vitamins (400 mg CaCl₂*2H₂O, 14 mg ZnSO₄*7H₂O, 16 mg MnSO₄*H₂O, 50 mg FeSO₄*7H₂O, 500 µg Thiamine, 500 µg Riboflavin, 500 µg Panthotenate, 500 µg Nicotinic acid, 500 µg Pyridoxaminiumdichlorid, 50 µg Biotine, 50 µg Cyanocobalamin, 50 µg Folic acid), and (iv) complete agar medium containing the target host
M. melolontha (CAM HM): prepared like CAM, but yeast extract was replaced by 1 % (w/v) of homogenised and dried M. melolontha larvae debris. For all experiments, petri dishes contained ten millilitres of the respective medium.

**Morphological characterisation and evaluation of conidiation**

Ten days post inoculation, the fungal colonies (FC) of each sub-cultivation were evaluated on the basis of morphological properties and colony diameters. Conidial yield of colonies was quantified and calculated per cm²: FC were punched out from agar plates, transferred into FALCON™ tubes containing 15 mL of 0.1 % (w/v) Tween 80 and homogenised using an Ultra-Turrax. Conidial concentrations in the suspensions were determined using a haematocytometer.

**Bioassays**

Conidia from the first and the fifth sub-cultivation were harvested and tested for their virulence against third instar larvae of M. melolontha. Larvae were collected from soil in Kötschach-Mauthen, Carinthia, Austria and quarantined for at least five weeks before usage. Bioassays were performed following the BIPESCO standard operation protocol (Nielsen et al. 2002): Larvae (15 per treatment) were dipped for five seconds into a defined conidial suspension (1*10⁷ mL⁻¹ in 0.1 % (w/v) Tween 80). Excess liquid was removed using a paper towel and the larvae were placed individually into plastic cups containing moistened cellulose. Larvae dipped into 0.1 % (w/v) Tween 80 served as a control. Larvae were kept at 20 °C in the dark, fed weekly with a slice of carrot, and checked periodically for mortality.

Bioassays were also conducted using Tenebrio molitor larvae applying the same procedure, but only with conidia harvested after the first sub-cultivation. T. molitor larvae were fed with oat flakes.

**Carbon utilisation profile**

Conidia from the first and fifth sub-cultivation were characterised for their carbon utilisation profiles using BIOLOG™ SF-P2 and BIOLOG™ SF-N2 microtiter plates, which offer the possibility to screen for 128 different carbon sources in total. The microtiter plate test-system was prepared according to the manufacturer’s instruction, with the exception that inoculation was done with a suspension of already germinated conidia: 1 mL of pure conidial suspensions (for preparation see above; 3-5*10⁷ conidia mL⁻¹) was incubated in 20 mL of the respective liquid medium at 25 °C and 180 rpm until germ tubes were clearly visible. Germinated conidia were then separated from nutrient medium by centrifugation (15280 g, 15 min) and washed with distilled water. The BIOLOG plates were incubated for three days at a temperature of 25 °C and > 65 % r.h. Turbidity (at 590 nm) in each well was measured by using a TECAN™ Sunrise microtiter plate reader (n = 3). In the course of the preparation of the microtiter plate inoculum, the germination rate and germination speed of conidia were microscopically evaluated.

**Data analysis**

For bioassays, linear regression equation was used to relate pathogenicity of sub-cultivated B. brongniartii conidia to third instar M. melolontha larvae.

Data analysis of carbon utilisation patterns was performed by calculating the average of relative carbon utilisation, whereby the absorption of the reference well was subtracted from the values measured for each well containing a defined carbon source. The second highest absorbency of each attempt, respectively, was set to 100 %. These data were grouped into four categories, which are defined as follows: group 1 < 10 %; group 2 < 40 %; group 3 < 70 %; group 4 > 70 % carbon utilisation. The data of absolute utilisation of carbon sources (OD₅₉₀ minus OD₅₉₀ of the reference well) were statistically analysed by student's t-test.
Results and discussion

Morphological characterisation and evaluation of conidiation

*B. brongniartii* showed variations in morphology and colony diameters (growth rate) in respect to different nutrient media. Differences in conidia production calculated per cm² were not observed. For all three batches, the typical colony forms when grown on S2G could be described as fleecy, on CAM colonies were flat and velvety, on CAMm rather wooly and on CAM HM very powdery. Colonies were white to cream-coloured, on S2G they turned lightly yellowish. On the chemically defined CAMm, the fungus grew slower but produced adequate biomass, what makes this synthetic medium interesting for physiological studies with *B. brongniartii*.

Up to the fifth sub-cultivation no changes in morphology and conidiation on the four media could be found, with one exception: In the batch derived from liquid nitrogen and re-cultivated on CAM, after the fifth passage, single colonies got fluffy and their conidial production declined. With any further sub-cultivation altered colony forms appeared in all three batches. The highest alterations were observed in the batch derived from liquid nitrogen, fewest changes in the batch which was passed through the target host. In respect to media types, on CAM the morphology of colonies changed most frequently.

Bioassays

Based on the current study it could be verified that conidia of all three batches of strain BIPESCO 2 were still aggressive to *M. melolontha* after five sub-cultivations on artificial media (Fig. 1). This observation is confirmed by Strasser and Pernfuss (2005). The authors reported that there are no significant differences in virulence against *M. melolontha* between deep frozen isolates of *B. brongniartii* (including strain BIPESCO 2) and the respective control strain directly isolated from *M. melolontha*. Even after four in vitro passages on S2G-agar (simulating the production line) none of the isolates showed any loss of virulence. Concerning the virulence of the production strains IMBST 95041 (BIPESCO 2) and IMBST 95031 reisolated from MELOCONT®-Pilzgerste, no negative impact of sub-culturing during production process could be determined.

Furthermore, in a long-term sub-culturing study with the *B. brongniartii* strains IMBST 94061 and LST 95024 Strasser and Pernfuss (2005) showed that successive transfers of up to six times on S2G-agar, Larva extract agar (EA) and Chitin peptone agar (CiP) were possible without a detectable loss of virulence. With any further passage a significant decrease of virulence could be observed, whereas virulence of the strains was stabilised best when cultivated on CiP and less when cultivated on EA.

In our present study all four nutrient media proved as suitable to maintain virulence of the fungus up to the fifth sub-cultivation (Fig. 1). The standard medium S2G and CAM HM showed uniform results in all three batches. The regression line relating virulence of conidia derived from liquid nitrogen and sub-cultivated on CAM represented the lowest gradient (k = 0.79). This can be correlated with the fact that the corresponding passage showed already altered colony forms (i.e. colony forms with a decreased conidial concentration). It is likely that the conidial suspension prepared for bioassays contained a mixture of highly virulent and already attenuated conidia. Consequently, the concentration of highly virulent conidia was less than 1*10⁷ mL⁻¹. It is necessary to verify this finding in further studies. Moreover, to check if virulence of the three batches becomes attenuated with further sub-cultivation and to prove whether differences become apparent in virulence stabilisation between the four nutrient media, bioassays will be repeated with conidia harvested after the tenth sub-cultivation (results expected in spring 2007).
A prove was carried out if *Tenebrio molitor* could be used as an alternative target to *M. melolontha* in virulence studies. *T. molitor* larvae can be purchased in pet shops, so it is not necessary to rear hundreds of individuals and to collect them outdoor as it is required for *M. melolontha* larvae. Our studies showed that bioassays according to the BIPESCO standard operation protocol are not suitable for *T. molitor*. Larvae suffer under the circumstances optimised for fungal growth and so there was a high mortality rate not only of *Beauveria* inoculated larvae, but also in the control. Even if most of inoculated larvae got mycosed with *B. brongniartii*, it was not certain, whether death was caused by the fungus or by other factors. Therefore, a modification of the standard procedure protocol for *T. molitor* bioassay is required.
Carbon utilisation profile

Significant changes in the degree of metabolising specific carbon sources were recorded utilising highly virulent and attenuated subcultures of *B. brongniartii* strains (BIPESCO 2 included). In fact the carbon utilisation profile was rather strain specific, but there were some carbon sources which were differently metabolised by all tested isolates when they were attenuated (Abendstein *et al.* 2000). The authors stated that carbon sources such as D-ribose, D-melibiose, D-melezitose, D-serine, L-erythriol, α-keto glutaric acid, γ-amino butyric acid and/or their respective enzymes could serve as virulent determinants and the BIOLOG<sup>TM</sup> test-system could be used for rapid strain characterisation and quality control of BCAs.

The current study aims at the confirmation of these data. In addition to the data currently under statistical evaluation, carbon utilisation profiles will also be examined after the tenth sub-cultivation and correlated with the data from the corresponding bioassay. This will answer the questions whether (i) attenuated forms of *B. brongniartii* may be detected using carbon utilisation profiles, (ii) the switch of highly virulent strains to vegetative growth can be monitored, and (iii) to which extent media types can minimise such changes. Currently, data are under evaluation and will be published after the completion of experiments.

References


