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Biology, Monitoring and Management of Economically Important Wireworm Species (Coleoptera: Elateridae) in Organic Farming

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Abstract

Wireworms, the larvae of click beetle species *Agriotes lineatus*, *A. obscurus* and *A. sputator* are serious pests with increasing importance for several field crops. Since synthetic insecticides are prohibited in Organic Farming, indirect control approaches e.g. cultural or biological methods have to be used in organic crop production. Both biological and cultural measures heavily depend on extensive knowledge on the biology of a given pest. In our study we focused on the biology of important click beetle species occurring in Germany and on the efficacy of sex pheromone traps. The biology was studied in vials at constant temperature of 20°C (*A. obscurus*) and in rearing cages under semi-natural conditions (*A. lineatus* and *A. obscurus*) both focusing on larval morphology and development time. The efficacy of pheromone traps for male click beetles was tested with respect to the range of attraction (mark-release-recapture method) and to mass trapping over a five years period expected to reduce soil wireworm abundance. Finally, a strain of *Beauveria bassiana* was tested in the laboratory and in small-plot field experiments for efficient biological control of wireworms. All experiments were conducted at the experimental farm for Organic Agriculture 'Wiesengut' at Hennef/Sieg, Germany, belonging to the University of Bonn.

A minor part of the larvae grown in vials at 20°C already transformed into adults in the second year after egg-laying, completing their life cycle (from egg to adult) in 14 months with only one overwintering and two calendar years. Most of the larvae entered into a third year of development. In laboratory (vials) between 8 to 11 larval instars were recorded, even when sufficient soil moisture and food were present. Under semi-natural conditions 13 larval instars for *A. obscurus* and 12 for *A. lineatus* were observed to-date. The average number of *A. obscurus* instars after completing the first year of development in the field was 4.7 compared with 6.2 under laboratory conditions. At constant temperature (20°C) the life cycle of *A. obscurus* was completed in 841 days corresponding to 9,248 degree days (above a base of 9°C).

Experiments on the range of attraction showed that 40% of the released beetles (*A. lineatus* and *A. obscurus*) were recaptured. Males of both species were recaptured from all release points and the percentage recapture decreased (in part significantly) with increasing distance from 76% (2 m) to 35% (15 m) and 9% (60 m), respectively. In a long-term mass trapping experiment of over 5 years, pheromone traps in a grass-clover ley attracted a total of 12,378 male adults of three *Agriotes* species. The adult swarming period lasted from late April to late August with one major and a small peak in the successive years. The leading trapped species was *A. lineatus* followed by *A. obscurus* and *A. sputator* respectively. Overall 431 wireworms exclusively consisting of *Agriotes* species were captured, with 165 individuals for *A. obscurus*, 162 for *A. lineatus* and 15 for *A. sputator* and no significant difference between pheromone treated and untreated plots. Damage assessment experiments carried out after five years removal of male adults showed no subsequent wireworm potato impairment difference in treated and untreated plots. Application of *B. bassiana* under laboratory conditions showed a significant mortality in high number of wireworms boxes (50%) compared to low number of wireworms (17%) and untreated boxes (13%) respectively. In field experiments no significant differences were noted between furrows and whole surface applications and the control treatments.

According to our findings on the biology of click beetles the two years following the year of oviposition are critical for potato tuber injuries. During that period crops should be grown where new oviposition is low, e.g. crops that are intensively hoed such as fababean, maize or field vegetables. A reduction of soil wireworm abundance and tuber injuries via pheromone mass trapping is apparently not possible. Biological control with entomopathogenic fungi requires further research.

Kurzfassung

Drahtwürmer, die Larven der Schnellkäferarten *Agriotes lineatus*, *A. obscurus* und *A. sputator* sind Schädlinge mit steigender Bedeutung für mehrere Kulturpflanzenarten in Deutschland. Im Organischen Landbau sind synthetische Insektizide nicht zugelassen. Daher ist die Regulation des Drahtwurmes im Organischen Landbau nur mit indirekten Ansätzen, z.B. kulturtechnischen oder biologischen Methoden, möglich. Sowohl biologische als auch kulturtechnische Strategien basieren auf fundierten Kenntnissen der Biologie des betreffenden Schädlings. In der vorliegenden Arbeit liegt der Fokus auf der Lebensweise wichtiger in Deutschland vorkommender Schnellkäferarten und auf der Effizienz von Sexualpheromonfallen. Die Reproduktionsbiologie der Schnellkäfer wurde in Glasflaschen bei einer konstanten Temperatur von 20°C (*A. obscurus*) und in Anzuchtkäfigen unter naturnahen Bedingungen (*A. lineatus* und *A. obscurus*) untersucht. Es wurden Parameter der Morphologie und Entwicklungszeit der Larven erfasst. Die Effizienz der Pheromonfallen für das Fangen männlicher Schnellkäfer wurde im Hinblick auf den Attraktionsradius (*mark-release-recapture method*) und im Hinblick auf die Anzahl gefangener Käfer über eine Zeitspanne von fünf Jahren mit der Erwartung einer verringerten Drahtwurmabundanz geprüft. Zudem wurde die biologische Kontrollwirkung eines Stammes von *Beauveria bassiana* im Labor und in kleinparzelligen Feldversuchen geprüft. Alle Versuche wurden auf der Lehr- und Forschungsstation für Organischen Landbau ‚Wiesengut' der Universität Bonn in Hennef/Sieg durchgeführt.

Ein geringer Prozentsatz der bei 20°C in Glasflaschen herangezogenen Larven entwickelte sich schon im zweiten Jahr nach der Eiablage zu Adulten, das heißt, sie vervollständigten ihren Lebenszyklus (vom Ei zum adulten Tier) in 14 Monaten mit nur einer Überwinterung in zwei Kalenderjahren. Die meisten Larven durchliefen ein drittes Entwicklungsjahr. Im Labor (Glasflaschen) wurden zwischen 8 und 11 Larvenstadien beobachtet, auch bei hinreichender Bodenfeuchte und ausreichendem Nahrungsangebot. Unter naturnahen Bedingungen wurden 13 Larvenstadien für *A. obscurus* und 12 Stadien für *A. lineatus* erfasst. Die durchschnittliche Anzahl der Larvenstadien für *A. obscurus* nach Ende des ersten Entwicklungsjahrs im Feld betrug 4,7, im Vergleich zu 6,2 unter Laborbedingungen. Bei konstanter Temperatur (20°C) war der Lebenszyklus von *A. obscurus* nach 841 Tagen bzw. 9.248 Gradtagen beendet (über einer Basis von 9°C).

In Versuchen zum Attraktionsradius wurden 40% der freigesetzten Käfer (*A. lineatus* und *A. obscurus*) wiedergefangen. Männliche Exemplare beider Arten wurden von allen Freisetzungspunkten wiedergefangen. Der Anteil wiedergefangener Tiere nahm mit zunehmender Entfernung z.T. signifikant ab; von 76% (2 m) auf 35% (15 m) und 9% (60 m). In einem Langzeit-Massenfangversuch über fünf Jahre lockten in Kleegras positionierte Pheromonfallen insgesamt 12.378 männliche Adulte der drei *Agriotes*-Arten an. Die Schwarmperiode der adulten Tiere dauerte von Ende April bis Ende August mit einem größeren und einem kleineren Peak in den aufeinander folgenden Jahren. Die meisten der gefangenen Individuen gehörten der Art *A. lineatus* an, gefolgt von *A. obscurus* und *A. sputator*. Insgesamt wurden 431 Drahtwürmer, ausschließlich der Art *Agriotes* angehörend, gefangen, mit 165 Individuen für *A. obscurus*, 162 für *A. lineatus* und 15 für *A. sputator*. Zwischen Flächen mit und ohne Pheromonfallen bestand kein signifikanter Unterschied. Versuche zur Erfassung des drahtwurmverursachten Schadens an Kartoffeln, die nach fünf Jahren Pheromonfalleneinsatz durchgeführt wurden, zeigten keine signifikanten Unterschiede zwischen Parzellen mit und ohne Pheromonfallen. Die Anwendung von *B. bassiana* wirkte unter Laborbedingungen bei Ausbringung einer großen Anzahl Drahtwürmer in Boxen signifikant letal (50%), im Vergleich zum Besatz mit wenigen Drahtwürmern (17%) und unbehandelten Boxen (13%). In den Feldversuchen wurden keine signifikanten Unterschiede zwischen der Behandlung mit *B. bassiana* (Reihe bzw.Fläche) und der unbehandelten Kontrolle festgestellt.

Den vorliegenden Ergebnissen zur Lebensweise der Schnellkäfer zufolge sind die zwei Jahre nach dem Jahr der Eiablage kritisch für Schädigungen der Kartoffelknolle durch Drahtwürmer. Während dieser Periode sollten für die Eiablage wenig förderliche Kulturen mit intensiver mechanischer Unkrautregulation angebaut werden, wie z.B. Ackerbohnen, Mais oder Feldgemüse. Eine Reduktion der Drahtwurmabundanz im Boden bzw. der Knollenschädigung durch Massenfang mit Pheromonfallen erscheint nicht möglich. Biologische Regulierungsmaßnahmen mit entomopathogenen Pilzen bedürfen weiterer Forschung.

1 Introduction

Insect pests have been identified as a major constraint for increased productivity, especially for high-value crops and considered as serious threats to farmer's efforts. Insects are the most numerous and most diverse animals on earth and occur virtually in every aquatic and terrestrial habitat. Insects pollinate, parasitize, predate, defoliate, scavenge, promote decomposition and provide food for vertebrates. Actions taken to control insect pests by using synthetic chemical pesticides can affect many components of the environment (HAJEK 1997). Arthropod pests (belong to different orders such as Acari, Diptera, Thysanoptera, Homoptera, Coleoptera and Lepidoptera), weeds, animals and diseases cause global pre- and post-harvest losses for approximately 20-40% of potential production depending on the crop (OERKE et al. 1994, FAO 2005). The beetles or Coleoptera are the largest order, including 350,000 species (ARNETT JR. 1968) known to science, more than in any other order not only in the class of insecta, but also in the entire animal kingdom (Animalia). The click beetles (Elateridae) form a very distinct group in Coleoptera, and are the largest family in the Elateroidea with some 400 genera and 9,000 species worldwide (LAWRENCE 1982). There are about 150 species of click beetles recorded in Central Europe (FURLAN & TOTH 1999) known to be economically important. According to a recent survey in Rheinland-Pfalz, Germany, three species of wireworms, *Agriotes lineatus* (L.), *Agriotes obscurus* (L.) and *Agriotes sputator* (L.), are commonly associated with damage to a range of crops and live both in agro- and natural ecosystems (BURGHAUSE & SCHMITT 2011). Adult click beetles emerge from overwintering cells in early spring, and after mating females lay eggs singly or in clusters just below the soil surface, preferentially in locations such as leys (FOX 1973). Click beetles have similar life cycles featuring a prolonged period spent as larvae (2-5 years depending on the species) in the soil prior to pupation. The newly hatched larvae feed on seeds and seedlings of a wide range of crops such as sweet potato (CHALFANT et al. 1993), cereals (BLOT et al. 1999, FURLAN 2004), sugar beets, carrots and other vegetables (MILES 1942). Significant losses in crop yield, quality and marketability have been attributed to wireworms, a pest of growing concern. Potatoes are the most important root crop in Organic Agriculture and are particularly susceptible to this pest, and even comparatively low populations (<100,000/ha) in the field can cause economic losses (PARKER & HOWARD 2001). Potato tuber damage caused by wireworms is an increasing global problem with significant crop losses (5 to 25%) noted in North America (JANSSON & SEAL 1994). Likewise, in North Rhine-Westphalia (Germany), a minimum of 11% to a maximum of 80% of organic potato tuber harvest are prone to be down-graded or rejected outright because of wireworm injury, resulting in substantial economic losses (SCHEPL & PAFFRATH 2007).

Historically, it has been difficult to control this pest because of its long life cycle and ability to survive in both agro- and natural ecosystems. Organic crops are particularly prone to

wireworm damage due to the lack of chemical management options using insecticides. In Organic Farming no direct control methods against wireworms are currently available. Indirect control strategies, including crop rotation, soil tillage practices and biological control methods, may help to reduce wireworm damages, but their efficacy is unreliable under different environmental conditions. The low predictability of wireworm damage but also practical limitations with respect to crop rotation design additionally weaken the competitiveness of indirect control measures, which in general play a key role in Organic Agriculture (MOHLER & JOHNSON 2009). Wireworms are considered cryptic due to their polyphagous diet, subterranean habitat, long life-cycle and incomplete knowledge of certain aspects of their ecology. These challenges have prompted the development of sex pheromone traps for this pest because they target the more accessible adult stage as a surrogate for larvae (TOTH et al. 2003). Historically, the use of pheromone traps has been successful within integrated pest management programmes and monitoring systems for other pests, e.g., pea moth (MACAULEY et al. 1985), pink bollworm (BAKER et al. 1991), tomato pinworm (JENKINS et al. 1991), European corn borer (LANGENBRUCH & LORENZ 1992) and codling moth (KNIGHT & LIGHT 2005).

Similarly, pheromone-mediated male confusion applications for lepidopterous pests resulted in yield growth and significant continuous decrease of the egg population in mating-disrupted areas (DOANE et al. 1983, HEGAZI et al. 2007). Pheromone trapping of adult males can give an indication of the local population of wireworms in the soil (PARKER & HOWARD 2001), is also sensitive enough to detect low-density populations, and is, therefore, effective for tracking invasive species in the establishment phase (EL-SAYED et al. 2006, LIEBHOLD & TOBIN 2008). Although most of the effective mass trapping studies have been carried out with lepidopterous pests, pheromone trapping of click beetles has been recommended in a similar way (OLESCHENKO et al. 1987, KUDRYAVTSEV et al. 1993) and the development of YATLOR funnel traps proved to be highly efficient in capturing click beetle (*Agriotes*) species (FURLAN et al. 2001a). Despite the proven performance of these pheromone traps, more information on the range of attraction of these traps, a clear relationship between male click beetle catches in pheromone traps and belowground wireworm distribution still needs to be addressed. Accordingly more knowledge on pheromone traps is needed before targeting mating prevention via mass trapping of males for successful practical implementation (BLACKSHAW & VERNON 2008). When establishing a trapping program, it is important to assess the effectiveness of trapping and explain the fluctuation of population density over time.

The entomopathogenic fungi (e.g. *Beauveria bassiana*) have been used as a biological control agent for wireworms with limited success. Biological control is generally perceived as providing both long-lasting insect control and having less potential for damage to the

environment or non-target organisms than chemical interventions (HOWARTH 1991, HOKKANEN & LYNCH 1995, GRACE 1997, KHETAN 2001). Before considering a biological control program, it is important to investigate the biology of this pest (wireworm) to interfere at a certain stage in its life cycle. In addition, studying oviposition, egg-laying behaviour and larval development leads to a better understanding of its biological and ecological characteristics which will determine a future control strategy, about which little is known in the literature.

The main objectives of this work can be summarised as follows:

- To estimate the adult and larval population levels, larval development and to identify the right timing for possible treatments through detailed study of the biology of *Agriotes* species.

- To investigate the behavioural responses of male click beetles to sex pheromone traps (Mark-release-recapture).

- To assess whether regular male mass trapping of three key *Agriotes* species over a longer period results in a decrease in soil wireworm abundance and subsequently in reduced damage in potatoes.

- To investigate the toxic effect of entomopathogenic fungus (*Beauveria bassiana*) on wireworm populations.

2 Literature review

2.1 Adult click beetle taxonomy and distribution (*Agriotes* spp.)

Click beetles are classified within the family Elateridae, which is contained within the super family Elateroidea in the suborder Polyphaga within the order Coleoptera. The family Elateridae is comprised of torpedo-shaped Coleopterans commonly called click beetles**,** elaters**,** snapping beetles**,** spring beetles or skipjacks. Elateridae is a large family with representatives in many places around the world. There are approximately 400 genera and 9000 species worldwide in the family, and more are continually being discovered every year (IVASCHENKO & ADAMENKO 1980, YATSYNIN et al. 1980, PARKER 1982, LAWRENCE 1982, KAMM et al. 1983, BORG-KARLSON et al. 1988). Adult click beetles can be found on flowers and plants and under the bark of trees. Adults can be collected on vegetation, but some click beetle species are also strongly attracted to light, where they can be collected quite easily. The adults do not cause any important damage; but their presence can give an indication of the local population of a larval stage known as wireworms, in the soil or in rotting logs (PARKER & HOWARD 2001). Many species of wireworms are destructive, feeding on the roots of plants and on freshly scattered seed. Some species whose larvae often inhabit the soil of farm land in enormous numbers are especially devastating.

There are different species of plant-damaging click beetles in Central Europe but the most important pest species in Germany are *Agriotes lineatus* (L.), *A. obscurus* (L.) and *A. sputator* (L.) (FURLAN & TOTH 1999). Adult click beetles are elongate, variable in size, somewhat flattened in appearance, and dark brown to chestnut brown in colour. The Elateridae family of beetles has several features that aid in their identification. Adults of *A. lineatus* are 8 to 10 mm long and 2.5 to 3 mm wide. The body is dark yellowish-brown and the head darker with moderate yellowish-grey pubescence. The antennae and legs are light brown. Elytra are 2.1 times as long as wide; sides slightly divergent to middle, then round to apex with paired striae. The pronotum subquadrate is relatively longer than wide; sides parallel to apical one-third, then round to apex with slightly divergent hind angles. The antennae extend to within one segment before or beyond the apex of the hind angle of the prothorax with the second segment 1.4 times as long as third while the second and third segments together are 1.8 times as long as the fourth. *Agriotes lineatus* is typically more slender than *A. obscurus*, which is broader across the abdomen. *Agriotes lineatus* is further differentiated by the presence of lighter brown ridges running parallel to the body along the surface of the elytra, from where it derived its name, the lined click beetles. Genitalia are elongate and deeply margined with a basal piece.

Adults of *A. obscurus* are 8 to 10 mm long and 2.5 to 3 mm wide. The body is uniformly dark brown, with a relatively large, densely punctured pronotum. The antennae extend to or up to

the length of one segment beyond the apex of the hind angle of the prothorax with the second segment 1.1 times as long as the third while the second and third together are 1.9 times as long as the fourth. The Elytra usually extend to the tip or near the tip of the abdomen and are 2.1 times as long as wide and lighter brown than the pronotum. The pronotum is slightly wider than long with sides subparallel or distinctly sinuate and widest at the apical one-third and rounded to the apex. Pubescence is moderate to conspicuous, greyish to yellowish-grey. Genitalia are very similar with those of *A. lineatus*.

Adults of *A. sputator* are 6 to 8.5 mm long and about 2 mm wide. The body is slightly robust, mainly reddish brown, with the pronotum somewhat darker and relatively shiny. The antennae extend to the hind angle of the prothorax or up to one segment beyond. The second segment is 1.1 times as long as the third while the second and third together are 1.7 times as long as the fourth. The hard front wings are 2.1 times as long as wide with the sides slightly divergent to the middle and then rounded to the apex. Genitalia are strongly curved and deeply margined with basal piece (ESCHSCHOLTZ 1829, WESTWOOD 1838, CANDEZE 1863, ROBERTS 1921, 1922, 1928, BROWN 1940, LANE 1952, EDIT 1953).

The click beetles have a flexible joint at the base of its elytra, a feature found in only a few species of Coleoptera outside this family. All click beetles have eleven (usually) serrated (saw-like) antennal segments (ARNETT JR. 1971, TRIPLEHORN & JOHNSON 2005). The beetle's head and consequent position of the mouthparts and an unusual spine and groove arrangement under its body enables it to click and jump away from danger (STIBICK 1979). When the click beetle suspects danger, it pulls in its legs and drops to the ground where it can hide among fallen leaves or in vegetation. This free-fall escape procedure often lands the click beetle on its back, a situation in which many insects are left completely helpless. When the click beetle falls on its back it lies still for a moment. Then utilizing its flexible joint, it arches its back until only its extremities are touching the ground. The click beetle then snaps its body straight, a movement that tosses the insect into the air in a series of acrobatic flipping. Often the click beetle will land on its back again, rest for a second, and then continue its snapping movement until it lands right side up. Each jump is accompanied by an audible click for which this beetle is named.

Adults emerge in late spring and early summer and are active for about 5 months and do not live for longer than 1 year; males emerge prior to the females and tend to die soon after mating (COHEN 1942). 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). *Agriotes* species flight behaviour seems to be trivial: flight movement is typically for short distances and for seeking food, locating mates, finding oviposition sites, escaping predation or escaping harsh environmental conditions (CHAPMAN 1975), although some large-scale directional movement

does occur in the field (BRIAN 1947, ROEBUCK et al. 1947, SCHALLHART et al. 2009, SUFYAN et al. 2011). Field studies suggest that there are interspecific differences in the estimated walking speed (per day) of male *Agriotes* species; *A. lineatus* is considered the fastest traveller followed by *A. obscurus* and then *A. sputator* (HICKS & BLACKSHAW 2008). According to BROWN & KEASTER (1986) females show a lower flight activity than males and do not travel far to find oviposition sites, indicating that infestation into neighbouring areas may take quite some time. Measuring beetle activity in a given locality should therefore indicate the presence of an established local population, thus contributing to an assessment of the overall risk of wireworm infestation. This additional information would be particularly valuable for fields where soil sampling or baiting techniques have failed to detect larval infestations.

2.2 Wireworm biology and life cycle

Elaterids tend to have comparatively long life cycles for insects. Female click beetles lay their eggs in May or June in central European climatic conditions. A creamy white to grey brown elliptic (0.5 mm diameter) eggs are deposited just below the soil surface, either singly or in small clusters, and usually within the protection of grass which reduces the risks of desiccation (PARKER & HOWARD 2001). There are some indications that oviposition is reduced on arable land compared with grass, possibly due to lack of soil cover at key times of oviposition, and female food preference may also be involved (ROBERTS 1921, COHEN 1942, GOUGH & EVANS 1942). Eggs hatch in four to six weeks after oviposition depending on temperature, and newly hatched larvae move downwards through the soil. Wireworms are whitish and approximately 1.5 mm long when they hatch, but grow to around 25 mm in length and turn a shiny golden brown colour (ROBERTS 1919). Wireworms are slender, hard-bodied, smooth and leathery, thus earning their common name of wireworms. There are three pairs of short legs behind the head, which is dark brown with biting mouthparts, and a smooth segmented body (ANON 1983). In *Agriotes*, the last abdominal segment of the body is narrowing, with two small dark spots on the upper surface, a distinguishing feature of *Agriotes* wireworms (GLEN et al. 1943). Wireworms are extremely difficult to distinguish morphologically on the species level, and are considered cryptic due to their subterranean habitat, polyphagous diet and long life cycle (RILEY & KEASTER 1981). Wireworms mature very slowly, passing through three to six instars each year (ROBERTS 1919, 1921, 1922, PILL et al. 1976). The time necessary to complete larval development may vary considerably between genera and individual sites, but *Agriotes* wireworms often spend 2 to 5 years in the soil before pupating (MILES 1942, PARKER & HOWARD 2001, FURLAN 2004). In *Agriotes* species, long periods of inactivity often precede a larval moult, and even after moulting, wireworms may remain inactive for some weeks (EVANS & GOUGH 1942). Many species

spend one or more winters in the larval stage, although a few species with shorter life cycles may overwinter as pupae or adults (FISHER et al. 1975). Wireworms may pass through 8 to 14 instars during their life cycle depending on species and climatic conditions. GORBUNOVA (1973) found that most *Agriotes* larvae moulted at a soil depth of up to 10 cm. The duration and intensity of the first mass moult depended on soil temperature and moisture content, while the second and third moults were dependent on soil temperature only (PARKER & HOWARD 2001). Generations overlap, so larvae of all stages may be in the soil at the same time. Wireworms usually reach maturity in July to September. Hence they burrow deep into the soil and hollow out small pupation cells 5 to 30 cm below the soil surface. After three to four weeks they become adults, but usually remain in the cell over winter or leave the cell to over winter in the soil (GRATWICK 1989).

Habitat preferences have been more thoroughly investigated than life history characteristics, perhaps because they are less time-consuming to study. There are two main periods of wireworm activity in central Europe, one from April to May and a second from September to October (PARKER & HOWARD 2001). According to CAMPBELL (1937) wireworm activity depends on specific soil moisture and temperature. Wireworm activity increases in dry substrates and decreases dramatically in very moist substrates, probably due to muscular inhibition (CAMPBELL 1937, LEES 1943a). When food is available there is less movement, possibly because food itself is a source of moisture. Wireworms prefer a soil moisture content of 9-12%, and experience fatality in extreme desiccated or saturated soil conditions for longer periods (CAMPBELL 1937, LEFKO et al. 1998a). Wireworms prefer a soil temperature range of about 10-25 degrees Celsius (CAMPBELL 1937, FISHER et al. 1975). They migrate vertically through the soil twice each year. The first migration occurs in spring when the upper soil surface temperature increases. In mid-summer the high soil temperature drives wireworms down the soil profile, a feature of wireworm behavioural ecology recognized as early as the 1920s (MCCOLLOCH & HAYES 1923). The second migration occurs in fall when the wireworms once again move upwards as temperature fall; then again return to deeper soil in late October for overwintering (FISHER et al. 1975). Wireworms move to a depth of 3-5 inches or less when the soil temperature ranges from 10-25°C and move to depths below 6 inches when the soil temperature rises above 25 degrees or falls below 10°C (FISHER et al. 1975). FURLAN (1998) found that the vertical distribution of *A. ustulatus* wireworms in northern Italy was dependent on soil temperature during autumn, winter and spring (late-instar larvae were found as deep as 60 cm), but mainly on moist soil during the summer. Extensive field studies have shown that the majority of larvae being found in the top 10 cm of soil probably reflect soil moisture and temperature conditions at the time of sampling (SEAL et al. 1992b, SEAL & CHALFANT 1994).

The range of distribution and apparent speed of movement were greater for older compared to younger larvae. Larger larvae are better able to disperse from the site of oviposition, leading to less aggregation with age (DOANE 1977). This could partly explain the patchy damage behaviour often observed within fields. SALT & HOLLICK (1946) found a non-random distribution of *Agriotes* larval population with very little to heavily infested areas in the same field. SCHALLHART et al. (2011) observed no movement of *Agriotes* larvae between cereal and grassland plots. This study might suggest that elaterid larvae rarely disperse between crops as long as local food supply is sufficient. According to DOBROVOLSKY (1970) & ARAKAKI et al. (2010), wireworms can cover distances up to 240 cm, with some directional movements at small scale from 10 to 20 cm depending on species, soils and cultivation type. Larval movements decreased with increasing soil moisture level and mortality increased to 80% in very moist soil (40-45%) after 5 weeks time (SEAL & JANSSON unpublished).

Agriotes larval feeding behaviour appears to be mediated by short distance mechanisms involving dietary preference (CHATON et al. 2003). According to SALISBURY & LEATHER (1998), wireworms lack efficient receptors that able to elicit a response to the food source attractivity, as also observed for other larval Coleoptera living in confined areas. In contrast to this apparent lack of a food quality detection system over long distances, the larvae seem to possess a gustative system for short-distance food choice, which may be localised on the mouthparts. Moreover, some studies suggest that carbon dioxide is not attractive to the larvae (CHATON et al. 2003), which is in contrast to previous studies in larval attractivity (DOANE 1961, AGUILAR 1962) which involved carbon dioxide released by root respiration. In addition to the forms of attraction above, wireworms appear to show preferences towards different parts of a field. RILEY & KEASTER (1984) found that wireworms are more likely to be found in fresh manure pats and the soil underneath them than in a decomposed manure pat or soil without manure. Certain fields are more susceptible to wireworm damage than others. RILEY & KEASTER (1984) suggest that crops planted in former cow pastures may be especially susceptible due to the increased numbers of wireworms under manure. Fields with native grasses and left fallow for 10 years make ideal habitats for elaterids and may be contributing to the problems observed in arable rotations (HANCOCK et al. 1992, PARKER 1999).

2.3 Wireworm damage to crops

Wireworms, the polyphagous larvae of click beetles (Coleoptera: Elateridae), are serious pests of many different crops all around the world (KLINGER & DOANE 1974, WIGHTMAN & MORRISON 1978, IVASHCHENKO & ADAMENKO 1981). *Agriotes* wireworms are predominantly herbivorous, although some *A. obscurus* larvae individuals feed on animal prey (TRAUGOTT et al. 2008). Crops susceptible to injury are cereals, maize, potatoes, sugar beet, strawberries and vegetables (STAMOPOULOS 1995), causing serious loss of plants if

attack occurs in the later stages of the crop after thinning (MILES 1942, ANON 1983). Autumn- and spring-sown oats and wheat are more susceptible than barley and rye, which are less likely to be injured (GLOGOZA 2001). Other crops such as *Brassicas*, leeks, beans, tomatoes and carrots are also attacked at both the seedling stage and in later development, although once established serious damage is rare (MILES 1942a, COCKBILL et al. 1945, GRIFFITHS 1974, ANON 1983). Wireworms primarily feed on underground parts, but the seriousness of the damage depends on a number of factors, including the plant species, growth stage, vigour when attacked, and plant density (ANON 1983). Damage caused by wireworms can result in substantial yield losses and damage to maize can be particularly serious due to its low plant density which may sometimes require crop replanting (SMITH et al. 1981, ANON 1983, FURLAN 1989, 1990, LEFKO et al. 1998a, 1998b, SIMMONS et al. 1998). In some cases up to 35% of maize crop might be lost due to wireworm damage (APABLAZA et al. 1977).

Generally high wireworm populations have been linked to long-term grassland fields (MILES 1942, ANON 1948), as this habitat is usually favourable for wireworm survival. Damaged plants can wilt and die, resulting in thin stands and bare spots may appear. Wireworms may also bore into the underground portion of the stem or feed the roots of the plant, resulting in withering and structural instability (KULASH & MONROE 1955, MUNSON et al. 1986, SIMMONS et al. 1998). Young wireworms smaller than 5 mm (length) are not considered to be capable of causing significant crop damage. As wireworms increase in age and size, they become more capable of destruction and are more readily seen in the soil (PARKER & HOWARD 2001).

The potato is the most important vegetable in Organic Farming and fourth (after rice, wheat and corn) most important agricultural crop worldwide that plays a key role in feeding the growing world population (FAO 2005). There are about 39 species of wireworms from 12 genera that have been recorded as important constraints for potato production and causing serious damage (JANSSON & SEAL 1994). Some of these species are widely distributed, while others are only of regional or local importance. Wireworms cause severe injury to potato tubers, leaving tunnels and small round holes on the surface, reducing crop quality rather than yield; and even low populations (<100,000 wireworms/ha) can cause economic damage (PARKER & HOWARD 2001). Injuries caused by wireworms may facilitate the penetration of *Rhizoctonia solani* (black scurf) into the tuber and assist the formation of drycore symptoms. According to KEISER (2007), drycore symptoms were 2.46 times higher for tubers with wireworm damage than for clean tubers, suggesting that wireworms are important factors for the formation of drycore symptoms on tubers. Cosmetic damage, such as pits, scars or holes, and narrow tunnels to the roots or tubers occurs, resulting in lower market value or total refusal by retailers (PARKER & SEENY 1997).

Typical crop losses in North America caused by wireworm damage range from 5 to 25% (JANSSON & SEAL 1994). However in the last couple of years wireworm damage has become an increasing problem, with damage occurring in fields in all arable rotations even without long-term grassland history (DEMMLER 1999, PARKER & HOWARD 2001). In an investigation under the federal Organic Farming program, an increase of wireworm damage in Germany was observed during the last few years (VUB-RING ÖKOLOGISCHER LANDBAU E.V. – ÖKORING 2009). The share amount of damaged potatoes was about 7%, and the damage to tubers reduced crop quality rather than yield. There is some evidence that crops following long-term set-aside (1-5 years green fallow) provide a suitable habitat for wireworm and this may be contributing to the problems observed in arable rotations (HANCOCK et al. 1992, PARKER 1999). Several factors play an important role in enhancing wireworm problems in potatoes. The most common factor associated with wireworm damage is the cropping history of the field. Potatoes are most vulnerable to wireworm outbreaks when they follow a favourable host in the rotation, such as fodder grasses (SCHEPL & PAFFRATH 2005). Other precrops known to favour wireworm outbreaks in potato rotations include cereals, green fallows, sugar beet and alfalfa (MCSORLEY et al. 1987, JANSSON & LECRONE 1991). Another important factor in enhancing wireworm problems on potato is lack of soil moisture. When soils dry out, wireworms presumably seek moisture from potato tubers. In general, the drier the soil, the greater the incidence of wireworm feeding on potato tubers (JANSSON & LECRONE 1991).
Apparently, wireworm damage may be confounded with slug damage as slug entrance holes are very similar to those made by wireworms. However, slugs often hollow out large cavities within the tuber flesh, whereas wireworms do not. Wireworm holes may also provide initial access for slugs, nematodes or other soil organisms such as millipedes or bacterial rots, further increasing the damage (GRATWICK 1989, PARKER 2005). Larvae of all stages feed periodically on potato tubers as each larval instar passes through different phases during development (FURLAN 2004). It has been found in various wireworm food preference studies (ROBERTS 1919, CHATON et al. 2003, TRAUGOTT et al. 2008), that *Agriotes* larvae preferred to feed on a mixed diet of weeds, cereals, potato tubers and even also seed flour materials. According to CHATON et al. (2003) larval feeding choice may also have been affected over short rather than long distances. The feeding pattern of *Agriotes* larvae appeared to be very sporadic and limited by temperature, relative humidity, and perhaps by endogenous factors. In agro-ecosystems, *Agriotes* larvae feed in the top 5 to 10 cm of soil, but they also migrate very deeply into the soil during extreme (winter and dry) periods, and larvae remain in the resting layer and move between the two layers with relative ease (CHATON et al. 2003).

2.4 Use of sex pheromone traps

Pheromones are natural chemicals produced in the body of an animal known to help in communicating with other members of its species (BEROZE & KYDONIEUS 1982). The first semiochemical to be chemically characterized was the sex pheromone of the silkworm moth (*Bombyx mori*) by BUTENANDT and coworkers in 1959. A considerable advancement has been made in chemical techniques and equipment to identify compounds from ever smaller amounts of material, and the field has progressed rapidly as a result of the identification of the first sex pheromone. The insects world is filled with many odours. Insects use these odours to cue them in a variety of complex social behaviours, including courtship, mating and egg laying. The pheromone-baited trap has become one of the most widely used methods to gather insects from a wide area and thus can be used to sample sparse insect populations (WALL 1989). Pheromone traps are typically very specific in attracting the target species, hence avoiding the need to sort out pests in the samples collected in nonspecific traps that require skill and considerable time. The monitoring of insect populations has long been recognized as an essential component of any successful pest control programme (PINNIGER 1989). With regard to insect pest management, population dynamics and long-term monitoring can help to elucidate patterns in population life cycles. These patterns can then be combined with the known biology of the insect to define parameters and strategies for an effective control campaign (NORTON & MUMFORD 1993). Pheromones can be sub-divided into different categories. For example, sex pheromones are chemicals which mediate interactions between sexes of the same species; most are produced by females and attract males, although some examples of male-produced pheromones are also known. Other types of pheromones include trail pheromones (which guide social insects to distant food sources), aggregation pheromones which may or may not be produced by both males and females to congregate the species for feeding or reproduction, alarm pheromones which may serve to rapidly disperse a group of insects, usually as a response to predation (NORDLUND 1981) and oviposition-deterring pheromones (which deter females from laying eggs in the same resource as another female). Most sex pheromones are not a single compound, but rather a blend of several compounds, with an accurate concentration and ratio to bring about the appropriate behavioural responses to a particular species.

Currently, the most widely used semiochemicals in pest management are the insect sex pheromones, particularly those of lepidopteran pests, which were amongst the first to be identified and synthesized (ARN et al. 1992). Recent progress in pheromone-monitoring traps have made them available for a wide variety of pests, including Coleoptera, Homoptera and Diptera in agriculture, horticulture, forests and other stored products (EL-SAYED et al. 2008). Pheromones have also been successfully applied for control and monitoring of pea moth (MACAULEY et al. 1985), spruce budworm (ALLAN et al. 1986), fall armyworm (ADAMS et

al. 1989), pink bollworm (BAKER et al. 1991), European corn borer (LANGENBRUCH & LORENZ 1992), tomato pinworm (CARDE & MINKS 1995), pine sawfly (LYYTIKÄINEN-SAARENMAA et al. 2001), codling moth (KNIGHT & LIGHT 2005) and for mass trapping of *Melanotus okinawensis* Ohira adult males as a control strategy in Japan (ARAKAKI et al. 2008). This method of pheromone trapping based on the principle that as the Elateridae only seem to disperse locally (albeit this has not yet been fully understood), the numbers of trapped adults can give a hint of the local population of wireworms in the soil (PARKER & HOWARD 2001). Sex pheromone traps have been used for male adult trapping of main *Agriotes* species in the Netherlands (ESTER et al. 2001), Italy (FURLAN et al. 2001b, TOTH et al. 2002), Hungary (TOTH et al. 2002), Canada (VERNON 2004), Croatia (IVEZIC et al. 2007), Portugal and Bulgaria (TOTH et al. 2008) and the UK (HICKS & BLACKSHAW 2008). The information collected from insect monitoring with pheromones has led to forecasting future outbreaks and devising targetted control measures for *Agriotes* species, thus reducing the application of pesticides (TOTH et al. 2002). Chemical composition for *Agriotes* species comprise a mixture of geranyl octanoate and geranyl butanoate in a 9:1 ratio for *A. lineatus*; geranyl hexanoate and geranyl octanoate in a 1:1 ratio for *A. obscurus* and geranyl butanoate for *A. sputator* (TOTH et al. 2003).

As monitoring tools, pheromones may be used to detect the migration of a specific pest into a particular area, as a post-application means of assessing the effectiveness of other control measures, or to improve either the timing of more labour-intensive monitoring methods (such as visual plant searches) or the timing of control tactics such as pesticide sprays. By far the majority of monitoring traps uses female sex pheromones, and hence traps adult males. However, it is very often the larval stage of the pest that is the most damaging, and against which control tactics are generally targeted. Pheromone traps are sensitive enough to detect low-density populations, and therefore, capturing adults may give an indication of belowground wireworm population for some species (BLACKSHAW & VERNON 2008). Significant differences regarding species responses to sex pheromone traps and trap spacing were noted in recent mark-release studies of *A. lineatus*, *A. obscurus* and *A. sputator* (HICKS & BLACKSHAW 2008, SUFYAN et al. 2011). Traps used for relative monitoring can be both mechanical, such as suction traps and pitfall traps, or attractant traps such as light or pheromone traps.

Different kinds of traps were developed for monitoring the insect populations, but they were not completely suitable for both flying and crawling species. The recent development of YATLOR traps proved to be highly efficient in capturing click beetle (*Agriotes*) species, and is suitable for all the species through all seasons (OLESCHENKO et al. 1987, KUDRYAVTSEV et al. 1993). Pheromone traps have been rapidly adopted as an alternative pest control strategy by using different techniques including mass trapping, mating disruption or attract-and-kill

(EL-SAYED 2006). For that purpose large amounts of synthetic female sex pheromones have been released, thereby causing males to become confused and unable to locate females. This method was first developed using female sex pheromones released from steel planchettes on stakes for the species *Trichoplusia ni* (GASTON et al. 1967). Mating disruption causes disorientation and communication disruption between the sexes, and thus delays, reduces, or prevents fertilization of females. Similarly by mass trapping or attract-and-kill, a reduction in the number of available males may also contribute to managing pest population levels, especially in *Agriotes* species, where the larvae are difficult to control with pesticides due to their subterranean life habit (EL-SAYED 2006, VERNON & TOTH 2007). Future practical use of pheromones and other semiochemicals depend on the availability of odorants that enable efficient manipulation of mate- and host-finding behaviour in insects and can be developed as a biological tool component in integrated crop management programs.

2.5 Wireworm management options

Infestation assessment

A good estimation of the wireworm infestation level of a field and the prediction of the likely level of damage are essential for an effective risk assessment method which must be part of a wireworm management strategy. The best means of dealing with wireworms is early detection of areas with large populations because infestations vary from year to year (SMITH et al. 1981). There may be considerable variation both within and between fields. Sometimes the past history of a field is a good indicator, especially if wireworms have been a problem in previous seasons. There are a variety of sampling and baiting methods available for determining wireworm population levels. Sampling is as simple as taking several soil cores from a field and extracting the wireworms by hand-sorting, liquid separation method or putting samples in funnels with wireworm collecting vials below (ANON 1948). The collected wireworms recorded and converted into specimens per hectare, provide an estimate of the field population and assist decision-making for future crops (YATES & FINNEY 1942). However, sometimes soil cores may not be the best means of wireworm detection, since the occasional absence of wireworms does not provide an indication of the wireworm population in the sampling area (PARKER 1994). A second type of trap found to be successful in attracting *Agriotes* larvae is the bait trap, considered an effective tool for determining the presence of wireworms before crop planting. Cereal-baited traps have been reported to be more effective than soil cores at detecting wireworms (PARKER 1996) and corn/wheat bait, in comparison to other bait types, is considered as being the most effective for estimating wireworm populations (SIMMONS et al. 1998). If just one wireworm per trap is found on average after one week there is a high probability of damage (RICE & SIMMONS 1999). According to BECHINSKI et al. (1994) on average four wireworms per bait trap have a high

probability of economic damage in potatoes. Leaving bait traps for longer periods of time results in more wireworms being trapped. Trapping may be affected by soil moisture and temperature, and decisions on bait trap results should be carried out with caution (PARKER 1996).

Control methods

Control of wireworms is complicated due to their long life cycle, subterranean habitat and overlapping generations. Due to the long life cycle (3-5 years) of larvae, long-term plans are needed through the combination of integrated pest management strategies suitable for sustainable farming. A variety of non-chemical preventive techniques (crop rotation, soil drying, flooding and cultivation) as well as curative techniques (resistant varieties, trap crops and biological control) has been integrated to reduce the wireworm population in the field.

Cultural

Cultural practices involve any cultivation or seeding exercises which will discourage and modify the soil environment to render it unsuitable for wireworms. Summer bare fallow along with frequent tillage can reduce pest populations, especially the eggs and pupae which are more susceptible to mechanical damage from cultivation (ANDREWS et al. 2008). Tillage is likely to be most effective in optimal soil conditions (early spring when temperature begin to warm up, and again in early fall) when wireworms are active and most dense in the upper soil layers (PARKER & HOWARD 2001). According to SALT & HOLLICK (1949) by ploughing wireworms can be killed directly or reduced by exposing them to predators, heat, low moisture and altering the soil structure, making it unsuitable for both wireworms and adult oviposition. SEAL et al. (1992a) observed a significant wireworm reduction from 1.75 to 0.2 per bait trap in ploughed plots compared to no change in unploughed control plots. Wireworm control was also reported in long-term cultivation in Czechoslovakia, Central Bohemia and Florida (DIRLBEK et al. 1973, MASLER 1975, JANSSON & LECRONE 1991). PIQUE et al. (1998) discovered that fields in Catalonia that underwent extensive tilling did not require the application of pesticides for wireworm control. Similarly, DIRLBEK et al. (1973) noted a 90 percent reduction in click beetle population after 5 years of cultivation. Crop rotation to non-host crops such as lettuce, alfalfa, sunflowers and buckwheat can reduce wireworm populations (GIBSON et al. 1958). Rotation with these crops should be done cautiously as wireworm infestation to some of these crops (e.g., alfalfa) has not yet been confirmed. Crops such as linseed, pea and bean are considered to be tolerant to wireworm damage even in heavily infested fields (COCKBILL et al. 1945, ANON 1948).

SCHEPL & PAFFRATH (2003) reported that carrots and field bean have been shown to work well as catch crops in organic rotations with potato, where infestation levels were significantly lower than in rotations in which an under-sown crop was used. Similarly in

another study SCHEPL & PAFFRATH (2005) observed significantly reduced wireworm damage in a rotation that included only one winter cereal when compared to a rotation with two winter cereals and a year of mixed grass-clover. Rotation planned with cruciferous plants, such as mustard and cabbage, contained glucosinolates hydrolysed to a variety of biologically active products that are potentially useful in the long run for the control of soil-borne pests (LICHTENSTEIN et al. 1964). FURLAN et al. (2004) found high *Agriotes* larval mortality in Italy after incorporating freeze-dried whole *Brassica juncea* plants at the rate of 18 ton/ha and various Brassicaceae seed meals at 3-6 ton/ha. Similarly, in another pot experiment FURLAN (2007) reported that *Brassica carinata* defatted seed meal showed promising results against wireworm damage to potato tubers.

Plant resistance to wireworms is an important component in many successful integrated management programs (STRICKLAND et al. 1962). PARKER & HOWARD (2000) found a slight difference in seven commonly grown potato varieties in the UK to wireworm damage. In contrast, KWON et al. (1999) observed significant differences in potato cultivars with respect to their relative susceptibility to wireworm damage. Several field experiments have shown that the presence of total glycoalkaloid (TGA) and sugar content in potato cultivars plays a key role in predicting wireworm susceptibility (JONASSON & OLSSON 1994, OLSSON & JONASSON 1995). According to OLSSON & JONASSON (1995) wireworm damage was negatively correlated with the concentration of total glycoalkaloid (TGA) and positively correlated with the concentration of reducing sugars. In contrast JOHNSON et al. (2008) found a weak relationship between glycoalkaloid content and resistance to wireworms. It is still unclear that high concentration of glycoalkaloids potato cultivar resistant to wireworms can be acceptable for human use due to their toxicity (PARKER & HOWARD 2001).

Field flooding is another non-chemical control method to reduce wireworm populations. This approach obviously has limited applicability for farmers not near to major bodies of water or in climates with short growing seasons. All stages of *A. lineatus* and *A. obscurus* wireworms were found to die more quickly in saline soil at high temperatures than at lower temperatures in other soil types, indicating that flooding during the summer months may provide more effective control (VAN HERK & VERNON 2006). A continuous flooding of six weeks during late spring and summer with water temperatures around 24 degrees Celsius can kill wireworms (HALL & CHERRY 1993) and also prevent egg-laying by adult click beetles. Flooding for wireworm control can be effective, but is a slow process and may not be practical in many ecological cases. More studies are needed to determine a relationship between negative effects of flooding on soil characteristics (texture, structure and minerals) and the rate of wireworm mortality during flooding. There are some indications that wireworm damage to potato tubers tends to steadily increase from about mid-August onwards, so planting a variety that can be lifted as early as possible will help reduce the

damage risk. According to a recent study in Germany, NEUHOFF et al. (2007) found increasing damage from early August to late September. While the trend was fairly consistent, it was statistically significant only at some sites. Depending on the variety early harvest may affect tuber yield or skin set. Similarly SCHEPL & PAFFRATH (2005) observed less tuber damage (8-50%) for late July or early August harvests compared with early or mid-September harvests when damage was 72-77 percent.

Chemical

If all other integrated pest management tactics are unable to keep an insect pest population below an economic threshold, then use of an insecticide to control the pest and prevent economic loss may be chosen. Chemicals to control wireworms need to be easily incorporable into the soil and persistent, particularly for crops that are attacked later in the season, such as potato. Insecticides for wireworm control include liquid and granular formulations, seed treatments and fumigants. Historically wireworms have been controlled by a variety of organochlorine and organophosphate insecticides (VERNON et al. 2001). Several trials with newer insecticides have been carried out in the USA using pyrethroids; fipronil and neonicotinoids, but so far have only given moderate suppression of wireworms (KUHAR et al. 2003). The organophosphates chlorpyrifos, ethoprop, fonofos, phorate and diazinon, when applied either pre-planting as a broadcast or at planting over the furrow give the best control of wireworms (KUHAR et al. 2003). However, the long-lasting nature of these chemicals in the soil (a characteristic that made them so efficacious against wireworms) resulted in limited applicability due to their toxicity to humans, non-target organisms and environmental reasons. According to HANCOCK et al. (1986) the use of organophosphates or carbamates has significantly reduced wireworm damage in potatoes. However, over the past decade results obtained with various organophosphate insecticides and application methods were inconsistent, even contradictory due to variations in soil type, wireworm pressure, and climatic conditions. GRIFFITHS & BARDNER (1964) found that the organophosphate thionazin is as good at controlling wireworms as the organochlorine aldrin, and there is evidence that it increases stands of barley. However, it is only partially effective in protecting potatoes (GRIFFITHS et al. 1969). ARNOLD (1981) found that the insecticide permethrin is effective in controlling wireworm damage while the insecticide fenvalerate is only moderately effective. More recently, the pyrethroid tefluthrin (JUTSUM et al. 1986), the newly identified thianicotinyl compound thiamethoxam (SENN et al. 1998), fipronil (a phenyl pyrazole) (FURLAN & TOFFANIN 1998, SHAMIYEH et al. 1999) and imidacloprid (ALBAJES et al. 2003) have become the most common chemicals used for control of soil insect pests.

There are several methods for applying insecticide during planting, including: in-furrow application, row-band application and seed treatment (ARNOLD 1981, MUNSON et al. 1986). Although some of the following chemicals are no longer in use, the insecticides carbofuran,

chlorpyrifos, and terbufos have been effective when applied in-furrow and the insecticides carbofuran, chlorpyrifos, ethoprop, fonofos and terbufos have been effective in row-band applications (MUNSON et al. 1986). Similarly applications in other work showed that either pre-planting broadcast treatment or in-furrow treatment at planting give either the most practical or most effective means of control (TOBA & TURNER 1979, TOBA & POWELL 1986, TOBA 1987). KULASH & MONROE (1955) found that a combination of the fertilizer-insecticide mixture and the soil treatment applied to corn seed produced the largest number of healthy plants. Chemigation (applying insecticides admixed with irrigation water) is an alternative method of pesticide application that is partially effective as other means, but costs less (CHALFANT et al. 1993). A broad-spectrum insecticide tested against non-*Agriotes* wireworms on a range of agricultural crops including potato (BURRAGE et al. 1967, CARPENTER & SCOTT 1974, TEETES 1976, TOBA & TURNER 1979, STEWART 1981, TOBA & POWELL 1986, ALLSOPP & RADFORD 1987, HORNE & HORNE 1991, ROBERTSON 1991, CHALFANT et al. 1992) has shown that organophosphate insecticides can reduce wireworm damage. However, care should be taken when applying results from non-*Agriotes* to *Agriotes*-infested fields (ARNOUX et al. 1974), as genera difference may affect insecticide efficacy.

Biological

Widespread use of insecticides is not generally worthwhile economically as some insect pests have become resistant and some non-target organisms are adversely affected. Additionally, environmental and health concerns have arisen. This shift in control strategy has caused increased interest in long-term management plans throughout the wireworm life cycle rather than during the production of susceptible crops. Wireworm sporadic field distribution (ARNOLD 1981, SMITH et al. 1981) and subterranean habitat make them difficult targets for predators and parasitoids, and using these biological controls rarely has a significant effect on reducing populations in the field. Other alternate entomopathogenic agents that can reduce the risk of wireworm damage include the fungus species *Beauveria* and *Metarhizium* (JANSSON & SEAL 1994). Both species are natural enemies of diverse terrestrial arthropods and are important regulators of host populations in terrestrial ecosystems (HAJEK 1997). There are over 700 species of entomopathogenic fungi (ROBERTS et al. 1991), of which *Beauveria bassiana* and *Metarhizium anisopliae* have been studied most extensively for their potential as natural control agents. Compared to other microorganisms, fungi are known to infect a broader range of insects including Coleoptera, Diptera, Homoptera, Hymenoptera and Lepidoptera (GREATHEAD & PRIOR 1990, WHITTEN & OAKESHOTT 1991, PRIOR 1992, 1997, STARNES et al. 1993). There is worldwide interest in the use of entomopathogenic fungi as biological control agents and they could be valuable tools in future research (KHACHATOURIANS 1986).

When spores of entomopathogenic fungi come in contact with the cuticle (skin) of susceptible insects, they germinate and grow directly through the cuticle to the inner body of their host. Here the fungus proliferates throughout the insect's body, producing toxins and draining the insect of nutrients, eventually killing it (BATEMAN et al. 1996). It may take 3-5 days for insects to die, but infected cadavers may serve as a source of spores for secondary spread of the fungus. Therefore, unlike bacterial and viral pathogens of insects, *Beauveria* and other fungal pathogens infect the insect with contact and do not need to be consumed by their host to cause infection. Once the fungus has killed its host, it grows back out through the softer portions of the cuticle, covering the insect with a layer of white mold (therefore the name white muscadine disease). This downy mold produces millions of new spores that are released to the environment. Conidia are persistent in the soil while short-lived on leaf surfaces exposed to sunlight. For best results, applications should be made during the early growth stages of the insect before much damage has occurred, as it may take several days for the insect to die. Speed of kill depends on the number of spores contacting the insect, insect age, susceptibility and environmental conditions. Fungi are highly dependent on surrounding climatic conditions and the limits of fungal growth range between 5-35°C respectively, with optima between 20-30°C. The relative humidity (RH) should be more than 90% during 8-10 hours a day (FLEXNER et al. 1986, MCCOY et al. 1988). There are several behavioural and environmental factors that may affect the infection of wireworms (KABALUK et al. 2007, KABALUK & ERICSSON 2007b), and for development of the fungus as a control agent the problems of targeting wireworms and production and formulation of the isolates need to be overcome (KABALUK 2007).

Entomopathogenic fungi have been used successfully as an effective biological control agent for wireworms in Canada (KABALUK et al. 2001), Switzerland (KELLER & SCHWEIZER 2001), and have also been noted to attack wireworm adults in Italy and UK (PARKER & HOWARD 2001). Similarly, KELLER (1994) found significant (73-100%) mortality of *A. sputator* species caused by the fungus *Zoophthora elateridiphaga* in his long-term grassland field experiments in Switzerland. ERICSSON et al. (2007) found that a synergistic effect of *M. anisopliae* and SPINOSAD® resulted in higher mortality of *A. lineatus* and *A. obscurus* in laboratory and subsequent field trials in British Columbia. Similarly, larval mortality of the house fly, *M. domestica,* was substantially high (58-100%) when *M. anisopliae* was used in combination with a sublethal quantity of spinosad (SHARIFIFARD et al. 2011). There have been numerous studies focused on the potential use of entomopathogenic fungi in combination with sublethal doses of organic insecticides against various insect pests (MCCOY et al. 1988); among those relevant to this study are the compatibility of *M. anisopliae* with sublethal doses of chlorpyrifos, propetamphos and cyfluthrin against the German cockroach (PACHAMUTHU & KAMBLE 2000), a combination of *imidiacloprid* and diatomaceous earth

with *B. bassiana* on mole cricket (THOMPSON & BRANDEBBURG 2006) and *M. anisopliae* in combination with sublethal doses of *imidiacloprid* on the subterranean burrower bug *Cyrtomenus bergi* (JARAMILLO et al. 2005). *Metarhizium anisopliae* grows naturally in soils throughout the world and is considered harmful to soil-borne pests, primarily beetle larvae, and non-toxic to beneficial insects. A significant amount of fungus over a long period of time is needed to produce high mortality (KABALUK et al. 2001), but *M. anisopliae* seed treatment has been shown to enhance crop yield and development (KABALUK & ERICSSON 2007a). *Metarhizium anisopliae* produces destruxins that cause paralysis, and insects die between three and fourteen days after infection, depending on species and size. On the surface of cadavers, spores are produced (WHITTEN & OAKESHOTT 1991, STARNES et al. 1993) which can subsequently infect other susceptible hosts (BATEMAN et al. 1996).

Similarly entomopathogenic *B. bassiana* has been tested in a wide range of pest control scenarios and has been successfully used in many countries. It has been a focus for commercial production for the last 20-30 years and has been used to control the Colorado potato beetle in the former USSR since the mid-1960s and pine caterpillars in China since the mid-1970s. More recently it has been registered in several European, North American and South American countries for the control of a wide array of pests, ranging from the European corn borer to greenhouse thrips (SHAH & GOETTEL 1999). While under suitable conditions, efficacy rates can exceed 90%, in many instances, a considerably lower level but longer-term suppression can be sufficient to prevent crop damage. FERRON (1981) reported that young larvae of the European corn-borer, *Ostrinia nubilalis* are more susceptible to *B. bassiana* than older larvae. In another study FRAGUES et al. (1994) observed a substantial reduction (57%) of foliage consumption by Colorado potato beetle larvae after two days application of *B. bassiana*. Similar findings of reduced wireworm population were noticed by ESTER & HUITING (2007) when treated with *B. bassiana* in the Netherlands. Many of the important pests have proven to be susceptible targets for *B. bassiana*, but its relatively high production costs and high application rates may render it economically impractical (HLUCHY & SAMSINAKOVA 1989, ADANE et al. 1996, RICE & COGBURN 1999, BOURASSA et al. 2001, LORD 2001, MEIKLE et al. 2001, PADIN et al. 2002). Although infection with entomophagous fungi appears to be relatively common and sometimes effective at reducing adult populations, the impact on subsequent larval populations is still unclear.

In addition to fungi, entomopathogenic (insect-parasitic) nematodes have been used as biological control agents for soil insect pests since the 1970s, but they have not been successful for wireworm control (EIDT & THURSTON 1995). The parasitic nematode *Steinernema carpocapsae* (Weiser) was shown to reduce damage caused by wireworms to sweet potato (*Conoderus* spp.) (CREIGHTON et al. 1968), and gave promising results when used in combination with resistant cultivars and/or an insecticide (SCHALK et al. 1993).

However, damage protection by the nematode was variable. Other studies suggest that wireworms are generally not very susceptible to entomopathogenic nematodes (TOBA et al. 1983); although a hint of natural parasitism with Mermithid nematodes has been reported (DOANE et al. 1973). Morphological features of the wireworm mouth and anus, such as dense, branched hairs in the spiracular orifice and preoral cavity, may be physical deterrents to nematode infection (EIDT & THURSTON 1995).

Any approach of controlling however has to be based on knowledge on the biology of click beetles. In our work we focused on the reproduction cycle of click beetles including a metric assessment of the larval stages as well as on the efficacy of pheromone traps. Since *A. lineatus*, *A. obscurus* and *A. sputator* are by far prevailing in Germany, the present research work was mainly targeted on these three species.

3 Materials and methods

The experiments were carried out to study the biology of three click beetle species (*A. lineatus*, *A. obscurus* and *A. sputator*) in order to discover potential areas of weakness where the life cycle or behaviour of the insect can be exploited to gain control of the pest. The biological study was conducted in the laboratory and semi-natural (rearing cages) conditions at different temperatures and plant species. Moreover, long-term field experiments on species-specific sex pheromone traps (mass trapping of male click beetles to estimate the maximum sampling range and the effective sampling area of traps) were carried out to reduce wireworm populations. Additionally, entomopathogenic fungus *Beauveria bassiana* (BalsamoVuillemin strain ATCC 74040) was used in the laboratory and in small-plot field experiments as an effective biological control agent for wireworm species.

Study site

The study was conducted at the experimental station Wiesengut (50°48´ N, 7°17´ E, 62 meter above sea level) that belongs to the Institute of Organic Agriculture at Rheinische Friedrich-Wilhelms-Universität Bonn, Germany. The slightly acid alluvial soils of the mixed farm with suckler cows are characterized by a loamy texture and are relatively rich in mineral elements. The farm has been under organic management since 1986. The crop rotation includes winter rye, grass-clover ley, potatoes, winter wheat, faba bean and spring wheat. The average pH of the arable land (0-30 cm depth) is 6.1 and of pastures 5.8 respectively.

Climatic conditions

It is well known that temperature influences insect behaviour, physiology and development (ZASLAVSKI 1988) and affects the population dynamics in agricultural ecosystems (STRAND 2000, BOMMARCO 2001). Weather data were recorded at the experimental station from 2004 to 2009. The climate was comparatively mild with an annual average temperature of 10.3°C and average annual precipitation was about 881 mm during the last five years. Mean monthly air temperatures of the peak flight periods (April, May and June) of adult click beetles were recorded during the study from 2004-2008 at the experimental station (Figure 1).

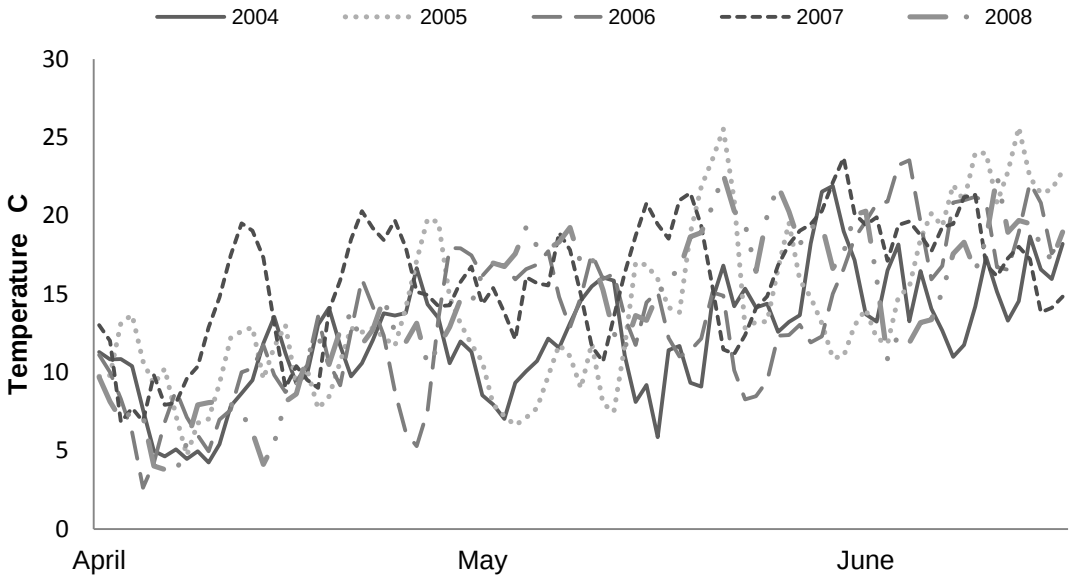

Figure 1: Mean air temperature pattern from April to end of June during the peak swarming period of *Agriotes* species at the Wiesengut in experimental years 2004-2008.

Mean sum of soil temperatures above 0°C (as frost inhibits insect activity) was calculated for the experimental years 2004-2009 except 2008 (Figure 2). The maximum sum of temperatures was noted in the months of July and August of successive years with a peak value of 659°C in July 2006.

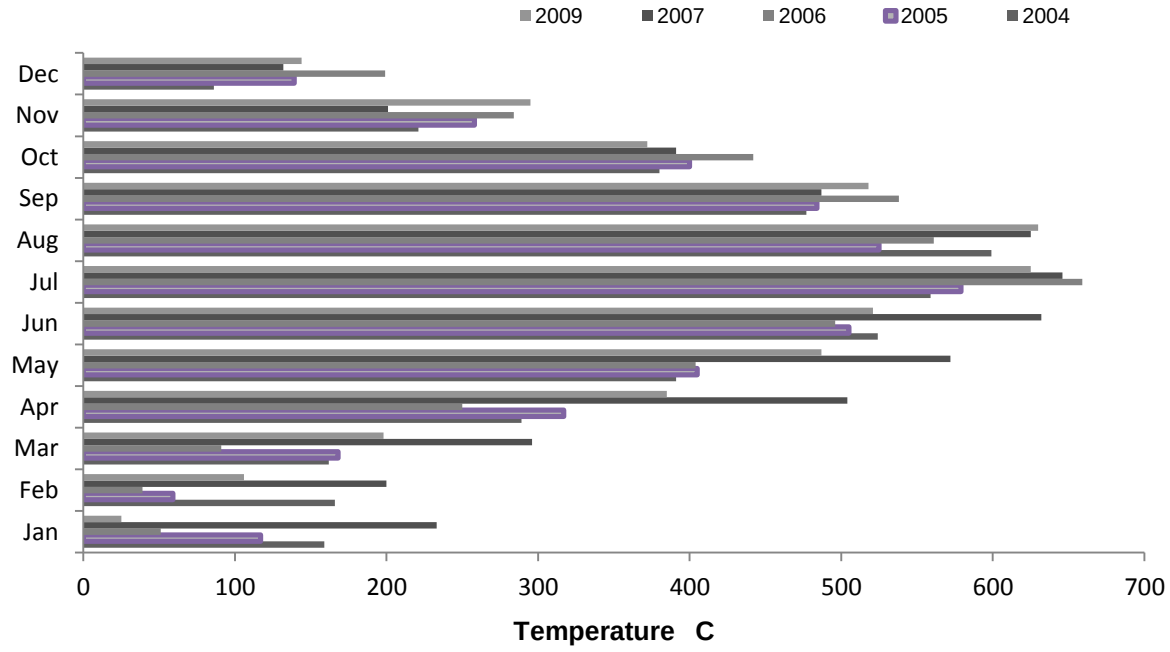

Figure 2: Mean monthly sum of soil temperature above 0°C during the experimental years 2004-2009 (except 2008).

Additionally we calculated the sum of soil temperatures above a base of 9°C (Figure 3) as no larval growth was observed below this temperature. A similar sum of temperature pattern was noted in July and August of successive years with a peak sum of 381°C in July 2006.

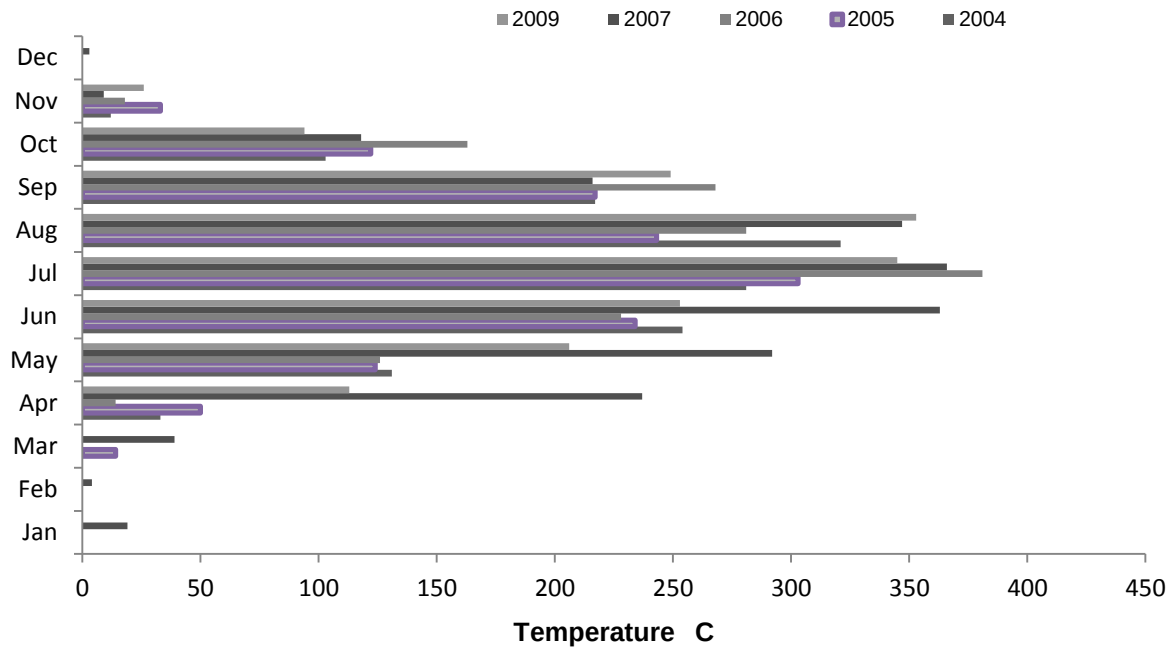

Figure 3: Mean monthly sum of soil temperature above a base of 9°C during the experimental years 2004-2009 (except 2008).

Precipitation data are shown in Table 1 for five months during the click beetles main activity period from April to August each year throughout the study. The maximum rainfall during the click beetles main activity period recorded was 515 mm in 2004 and a minimum of 355 mm in 2008. Further site details about weather can be found in HAAS (1995).

Table 1: Sum of rain at the Wiesengut during the main activity period of *Agriotes* species from April to August during 2004-2008.

∑ of rain in mm

	2004	**2005**	**2006**	**2007**	**2008**
April	56	95	60	0	71
May	87	81	123	125	20
June	80	25	55	110	115
July	113	201	35	98	96
August	179	100	120	139	53

3.1 Biology of *Agriotes lineatus* and *Agriotes obscurus*

In order to identify the right timing for possible treatments and implementation of effective integrated pest management strategies, the detailed biology of *A. lineatus* and *A. obscurus* was studied under laboratory and semi-field conditions. The study conducted in laboratory (vial test) was done with *A. obscurus* from 2009 to 2010 and in semi-natural conditions (rearing cages) with *A. lineatus* and *A. obscurus* from 2008 to 2010.

3.1.1 Laboratory experiment (vial test)

This experiment was carried from 2009 to 2010 in vials (2.7 cm in diameter and 9.2 cm high) maintained at constant temperature of 20°C with *A. obscurus*.

3.1.1.1 Adults and egg collection

Males and females of *A. obscurus* were collected in early spring (March-April) of 2009 by placing out forage traps made of 2×2 m plastic nets on bare soil in areas of the Wiesengut farm known to be infested with wireworms. The sheets were covered with fresh forage of *Lolium* and/or other Gramineae and Leguminosae species. Adult beetles congregated below the forage on the sheet and could easily be collected. Traps were inspected at least twice per week along with forage replacement. The collected beetles were sexed, identified (using a binocular microscope) and transferred to plastic boxes (18×12×9 cm high) containing dark moistened soil (half filled) and amended fresh Gramineae leaves. Groups of 5-10 females and 10-15 males were enclosed in each of 6-10 boxes (Figure 4).

Figure 4: Adult males and females inside boxes filled with moist soil and fresh Gramineae leaves, (Right): Plastic bags filled with moist soil and eggs.

The boxes were covered by a 2 mm mesh plastic net. The Gramineae leaves and soil were replaced and thoroughly screened for eggs using a binocular microscope every 2 days for 15-20 days. In some cases soil was replaced and screened every day to obtain newly-laid eggs. All eggs found were removed using a spoon and pincette and counted. The collected eggs were transferred to air-tight plastic bags together with moist soil for hatching (Figure 4). The plastic bags were kept at 20°C and observed every 1-2 days to ascertain the timing of egg-hatch and the percentage of hatched eggs. Forty newly hatched larvae were kept in vials (3 per vial) without food for a period of five weeks to examine their resistance to starvation.

3.1.1.2 Larval development

Newly hatched larvae (2-3) were put in half-filled plastic vials (2.7 cm in diameter and 9.2 cm high) with sandy moistened soil closed by an airtight plastic lid to retain humidity in the vials (Figure 5). Before the transfer of larvae, seeds (5-8) of *Lolium multiflorum* L. were put in to germinate inside each vial which was closed by an airtight plastic lid. Vials were kept at 20°C and inspected every 10-15 days for larval measurement with simultaneous replacement of soil and seeds inside. Larval development was examined by measuring head width, length and exuviae collection at every check by using DISKUS software microscopic system (see details in microscopic identification chapter). The life cycle was monitored until the beginning of November of each year (when the outside soil temperature decreased below 10°C). At this time (below 10°C) the larvae overwinter in natural conditions, and therefore the vials were shifted outside deep in the soil (60-80 cm soil depth) till the next spring.

Figure 5: Vials with grass seed germination and wireworms.

3.1.1.3 Larval overwintering

When the larvae had not pupated before winter, the vials were put into closed tubes deep in the soil (60-80 cm) to protect them from frost during the winter season. The temperature was recorded during the overwintering by putting universal serial bus data log deep in the tubes along with the vials. Vials were taken out in spring when soil temperature climbed above 10°C and transferred to climate chamber entering to next year development.

3.1.2 Installation of rearing cages (semi-natural conditions)

Two rearing facility cages were constructed at the border of an open field for the life-cycle study of *A. lineatus* and *A. obscurus* from 2008 to 2010. They were made of wood 1 m^2 ×1 m deep; open at the bottom to allow the soil to drain easily (Figure 6). The cages were closed at the top by a netted cage (1-2 mm mesh) 1×1×1 m high that allowed rain to penetrate. The cages were filled with common soil (from the area which had been dried in the open air for two months) and sand in a 1:1 ratio to remove wireworms. One cage was used for each species and each cage was separated into two parts; each half planted differently to the other half. The crop rotation in the cage was according to the local cropping system with maize and grass-clover (2008), mustard and grass-clover (2009) and wheat and grass-clover (2010) sown together during the life cycle study of both species. During dry (low rainfall) periods, the rearing cages were irrigated at least once a week to maintain a suitable soil moisture level in the upper part of the soil.

3.1.2.1 Introduction of adults into cages

Adult males and females of *A. lineatus* and *A. obscurus* were collected in early spring (March-April) of 2008 using sex pheromone and forage traps (2×2 m plastic nets) from a number of fallow and pasture fields in areas of the Wiesengut farm known to be infested with wireworms. Groups of (50-100) adult males and females per species were put into each cage every 5-10 days from early April to mid May depending on the number caught by the forage traps (see above for details). A total of at least 300-350 adults per cage were used. The eggs inspection was done periodically up from late April at different parts in cage between the plants by carefully turned over the upper soil layer.

Figure 6: Rearing cages.

3.1.2.2 Study of larval development

From June 2008 onwards, two soil samples (6 cm diameter × 25 cm deep) were collected per cage two times a month. The soil cores were hand-sorted to collect all wireworms present. The head-width of all collected larvae was measured and recorded at each examination time (see microscopic identification on page 33). All soil and larvae were returned to the cage. Soil samplings were replaced by two bait traps per cage from July 2008 onwards to avoid crop disturbance and to avoid killing the larvae while collecting wireworms for measurement (Figure 7). Each trap was made and used according to the description given by CHABERT & BLOT (1992); a modified version of the trap described by KIRFMAN et al. (1986). Bait traps consisted of a plastic pot 10 cm in diameter with holes drilled at the bottom; the pots were filled with vermiculite, 30 ml of wheat seeds and 30 ml of corn (maize) seeds. Cereal-baited traps have been reported to be equally or more effective than soil cores for detecting wireworms in most cases (PARKER 1996) and corn/wheat bait, in comparison to other bait types, is reported as being most effective at estimating wireworm populations (SIMMONS et al. 1998). The pots were wetted before being placed into the soil just below the surface and covered with an 18 cm diameter plastic lid placed a few centimetres above the rim of the pot. Traps were checked by hand-sorting and wireworms recovered were counted, identified and measured using DISKUS software microscopic system (see details in microscopic identification on page 33) before returning them to the cage. Traps inspections were done every 15 days with concurrent replacement of trap material. The larvae were assigned to the relevant instars based on the head measurements found in laboratory studies taking into account the standard deviation ranges. In cases of doubt, larvae were assigned to the instar class whose mean was closest to the actual head size.

Figure 7: Bait traps.

3.1.3 Microscopic identification

The magnifier used in the study was the DISKUS software microscopic system (www.hilgers.com), consisting of microscope, camera, high-resolution PC monitor with the program DISKUS (Figure 8). The DISKUS controls the conditions of the microscope which allows the program to perform measurements and to be used for microscopic documentation, i.e., recording, storage and reproduction of microscopic pictures. DISKUS provides the microscope user with the possibility of live observation at the PC screen or with a beamer. A high-definition digital camera JVC KY-F1030U was used to display the microscope insight images on screen with full resolution. The JVC KY-F1030U is a new-generation imaging camera with innovative features and functions, such as live 7.5 frames per second preview window, full-size (1360 x 1024) SXGA preview and an IEEE 1394 single cable solution. It has 1.45 million effective pixels and the C-mount allows a wide variety of lenses and scopes to be fitted to produce images with high resolution and excellent colour recognition. The JVC KY-F1030U is ideal for pathology applications for capturing images in microscopy, medical diagnostics and a wide range of other applications in agriculture. The identification of species (adults, larvae), separation of larval stages, egg numeration and calibrated measurements were done effectively with DISKUS and high-definition digital camera system in the current study experiments. Although it is possible to identify *Agriotes* larvae by the microscopic method, this method is problematic especially for the identification of the first instar wireworms using the available keys. Meanwhile molecular techniques have been developed for the identification of *Agriotes* species using a genetic analyser (ELLIS et al. 2009).

Figure 8: Digital image capture DISKUS-system (JVC KY-F1030U camera).

3.2 Range of attraction of pheromone traps (Mark-release-recapture)

In order to assess the efficacy of sex pheromone traps in attracting click beetles, a specific trial to estimate the range of attraction of pheromone traps was conducted by releasing tagged male click beetles at different distances from the trap. The adult stage is when the click beetle is most mobile, thus determining species range and rate of spread. Any control achieved before egg-laying has the potential to decrease the number of eggs that will give rise to the next generation of wireworms. It is therefore of practical importance to understand the biology of these pests to apply appropriate control measures that will be more efficacious. We expected to gain substantial information on the range of attraction of pheromone traps in the field space, allowing an estimation of the amount of traps needed to 'clean a field' and also assisting in making various control methods including mass trapping, mating disruption and physical exclusion. The experiment was carried out on two types of soil coverage (clover grass and bare soil) during natural dispersal peaks, i.e., May and June of successive years (2006-2008) with *A. lineatus* and *A. obscurus*.

3.2.1 Insect material

Adult male click beetles (*A. lineatus* and *A. obscurus*) were captured using Yatlor sex pheromone traps (FURLAN et al. 2001a) from a number of infested fields. All captured beetles were sexed, identified and placed in aerated boxes filled with moist soil and provided with fresh *Gramineae* leaves until the field experiments were established (maximum 14 days). Pheromones were provided by Csalomon (Plant Protection Institute, HAS, Budapest, Hungary) and comprised a mixture of geranyl octanoate and geranyl butanoate in a 9 : 1 ratio for *A. lineatus*, geranyl hexanoate and geranyl octanoate in a 1:1 ratio for *A. obscurus* and geranyl butanoate alone for *A. sputator* (TOTH et al. 2003, HICKS & BLACKSHAW 2008).

3.2.2 Marking and release of beetles

Different water proof colours (Edding 780) were used to paint the elytrae of the male beetles (Figure 9). One marking colour was used for each of the 12 treatments (six release distances = 2, 5, 10, 15, 20 and 60 m and two release directions from the trap (along and opposite to the known prevailing wind direction i.e. South-West). Actual wind directions were checked after the experiments based on daily averages at the farm's weather station. Data records confirm that the positioning of the traps was according to the prevailing wind direction. Mostly, the colour markings of the recaptured males were visible to the naked eye.

Figure 9: Marked male adults of *Agriotes lineatus*.

The release experiment was repeated in total five times over three vegetation periods (years) at different trial locations at least 150 m apart. Two YATLOR pheromone traps per trial including lures for either *A. lineatus* or *A. obscurus* were placed with a minimum distance of 150 m either in a permanent pasture field (grass-clover-ley: Trial 1, T3, T4 and T5) or in bare soil (arable land with potatoes: T2) with no wind obstacles in the field. In four trials twenty-five marked beetles of each species were released at each release point (Figure 10). In one trial (Trial 4), thirty marked beetles of each species were set free at each release point. Prior to release, beetles were transported to the experimental site in small vials allowing them to leave the vial on their own. After release, the pheromone traps were inspected after 10 minutes, 1 hour, 1 day, 3, 5, 12 and 30-36 days. In trial 3 the assessments had to be stopped after 20 days due to soil tillage for the subsequent crop. Trapped marked beetles were collected for identification and counted. The calculation of the percentage of recaptured beetles was based on the number of males that had left the release points. Dead beetles were replaced at the release point (1 hour after release) with extra living specimens. The experiment was terminated after 36 days.

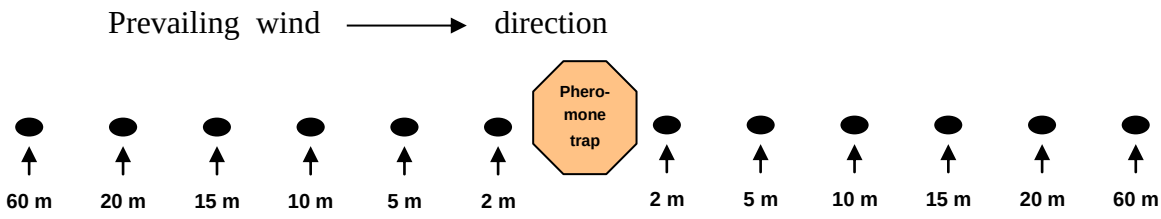

Figure 10: Experimental design of the release trials with *A. lineatus* and *A. obscurus* males.

3.3 Prevention trial

3.3.1 Experimental design

The study was conducted for five consecutive years (2004-2009) in a grass-clover ley known to have a high *Agriotes* population. Typically, high wireworm populations have traditionally been associated with long-term pasture fields (PARKER & SEENEY 1997), since these are preferred hosts for wireworms and are targeted oviposition sites for adult click beetles (FOX 1973). The purpose of the long-term study was to assess whether regular male mass trapping of three key *Agriotes* species over five years results in a decrease in soil wireworm abundance and subsequently in reduced damage in potatoes. The field chosen for study had been in temporary ley since 2003 and remained undisturbed (except grass cutting) until the planting of a potato crop in 2009.

Four equal-sized plots each one measuring 20 * 30 m = 600 m² were selected in the grass-clover ley following a pair-wise arrangement (Figure 11). On the Northern side two plots were supplied with pheromone traps at a distance of 50 m. On the Southern part of the ley two control plots without pheromone traps were established at a distance of 65 m to the pheromone traps. This distance was considered to be sufficient to avoid interferences since according to previous field studies walking is thought to be the main mode of adult click beetle dispersal (ROEBUCK et al. 1947), at least for short-range movements. Field studies also suggest that *Agriotes* males and females make mostly short journeys close to the place of emergence and this was ascertained by many recent findings, including our experiments on the range of attraction of pheromone traps, that have shown that the percentage recapture was very low (<10%) of click beetles released at a distance of 60 m to the traps (SUFYAN et al. 2011). For the three target species (*A. lineatus*, *A. obscurus* and *A. sputator*) single pheromone traps were placed randomly in the centre of the plot at a distance of one meter.

3.3.2 Pheromone traps installation

The Yatlor traps (FURLAN et al. 2001a) were used in this study to capture and confine adult male beetles (Figure 12), attracted to the internal pheromone lure and which fall in after ascending the shallow ramps. 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. No preservative was used in the traps. YATLOR traps are made of plastic in an Italian laboratory, and these traps proved to be suitable for catching both flying and crawling species all throughout the season (OLESCHENKO et al. 1987, KUDRYAVTSEV et al. 1993). Pheromones composition has already been described in the previous chapter on insect material on page 35. The traps were deployed in early April and sampling continued at approximately one-week intervals until August of each experimental year (2004-2009).

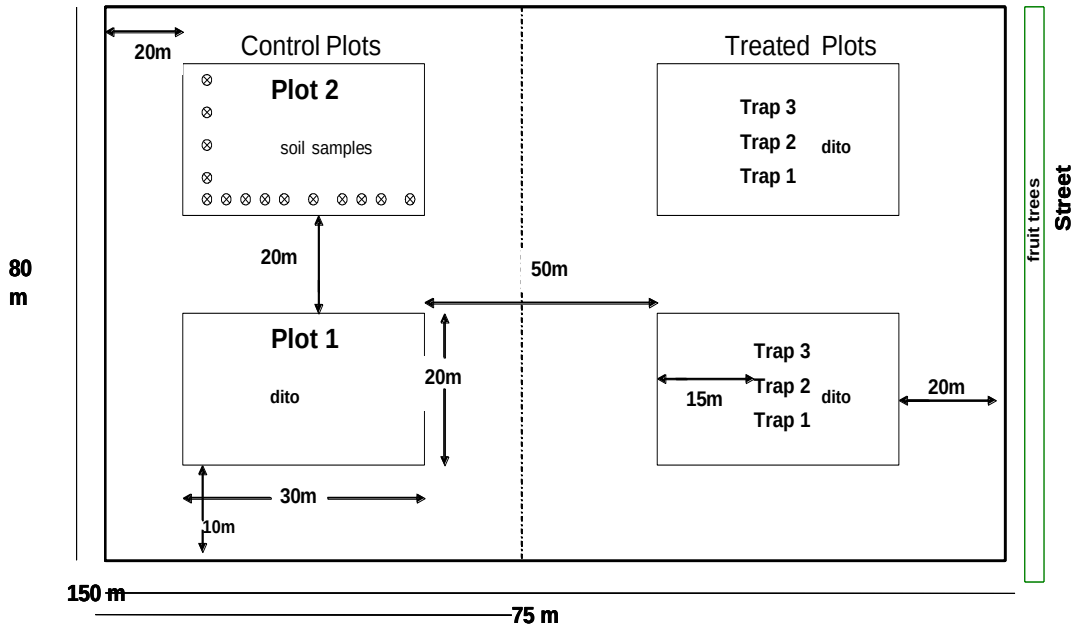

Figure 11: Experimental design of the prevention trial (2004-2009) on grass-clover ley, Wiesengut experimental station, Trap 1 = *A. lineatus*, Trap 2 = *A. obscurus* and Trap 3 = *A. sputator*.

Pheromone lures were replaced in intervals of 4-6 weeks. The trapped beetles were weekly removed from the traps and stored at -18°C for killing. The dead male beetles were counted and up from 2006 identified on the basis of pronotum and elytral characteristics (see details in Chapter 4). Calculations were done on the basis of captures per day. From the agronomic point of view the most important species responsible for the vast majority of wireworm attacks in Germany are *Agriotes lineatus*, *A. obscurus* and *A. sputator* (FURLAN & TOTH 1999, BURGHAUSE & SCHMITT 2011).

Figure 12: YATLOR sex pheromone trap on grass-clover ley, Prevention trial 2004-2009, Wiesengut experimental station.

3.3.3 Soil sampling and wireworm extraction procedure

Soil core samples were taken from both kinds of plots in 2004 and 2006 to 2009, once in the spring (May) and again in the autumn (September or late October), except 2006 when sampling was done three times. These sampling dates were chosen to coincide with peak activity and presumed feeding periods of larvae, which move lower down the soil profile during the extreme temperatures of summer or in winter (STALEY et al. 2007). Each plot was divided in-to four rows and each row was marked with 10 sampling points, with each point being about 2 m apart. Up to forty cores per plot (minimum 20) were sampled using a 13 cm diameter soil corer (26 cm depth) in a W-pattern giving a total of 160 cores in four plots except 2004 when a total of 200 cores were taken (Figure 13). From 2007 soil core sites were marked to observe the wireworm's exact capturing locations in treated and untreated plots to assess the range of the sex pheromone traps effectiveness. The outer 1 meter of each plot was not sampled to avoid edge effects.

Figure 13: (Left): Soil samples, (Right): Funnels with soil samples.

Each soil core was sealed in a plastic bag and labelled before being transported to the extraction place. All samples were initially hand-sorted for the presence of wireworms and any found wireworm was recorded and removed for identification. After hand-sorting, samples were placed in simple funnels (26 cm in diameter) provided with a 0.5 cm mesh at the bottom, with collecting vials under the funnels (Figure 13). The soil was allowed to dry for 4 to 8 weeks in a sheltered place to extract the remaining specimens. Larvae that fell into the collecting vials were counted and determined to species level as well as to larval stage by measuring the head capsule widths (KLAUSNITZER 1994, KAUPP & WURST 1997) by using DISKUS software microscopic system. The larvae of *A. lineatus*, *A. obscurus* and *A. sputator* (Figure 14) were described and illustrated many times by European worker (for example BELING 1883, FORD 1917, ROBERTS 1922, 1928, MESNIL 1930, EDIT 1954).

Figure 14: Click beetles larvae *A. obscurus* (left), *A. lineatus* (middle) *A. sputator* (right).

The larvae of *A. lineatus* are dirty yellowish-brown with a sparsely punctured body that grows to around 25 mm in length and about 2 mm in width. The first eight abdominal segments usually have small seta just anterior of the lateral anterotergal seta with sternal areas around coxae lined, accompanied by very small inconspicuous plates in membrane. There are three pairs of short legs behind the head, which is dark brown with biting mouthparts, and smooth segmented body (ANON 1983). There are some principal distinguishing characters of *Agriotes* larvae compare with other wireworm species such as *Melanotus*, *Limonius* and *Ampedus* spp. etc.; the presence of a tooth on the inner edge of the mandible and two eye-like pits near the base of the ninth abdominal segment, thought to be sensory organs (MILES 1942) (Figure 15). *Agriotes obscurus* larvae are apparently identical to *A. lineatus* but with slight differences in some characteristics. Some authors consider that *A. obscurus* larvae have the preapical tooth at an angle of 110 to 120 degrees with the apical portion of the mandible and the spiracles being slightly longer and narrower than those of *A. lineatus*, which have the preapical tooth at an angle of 60 to 90 degrees. The larvae of *A. sputator* can be distinguished from *A. lineatus* and *A. obscurus* by the presence of many small conspicuous plates on the intercoxal areas. Normally full-grown larvae reach about 17 mm in length and 1.5 mm in width with finely punctured anterior portion of mesothorax, metathorax and first eight abdominal segments. The larval ninth abdominal segment terminates rather acutely compared to other species with a blunt terminus.

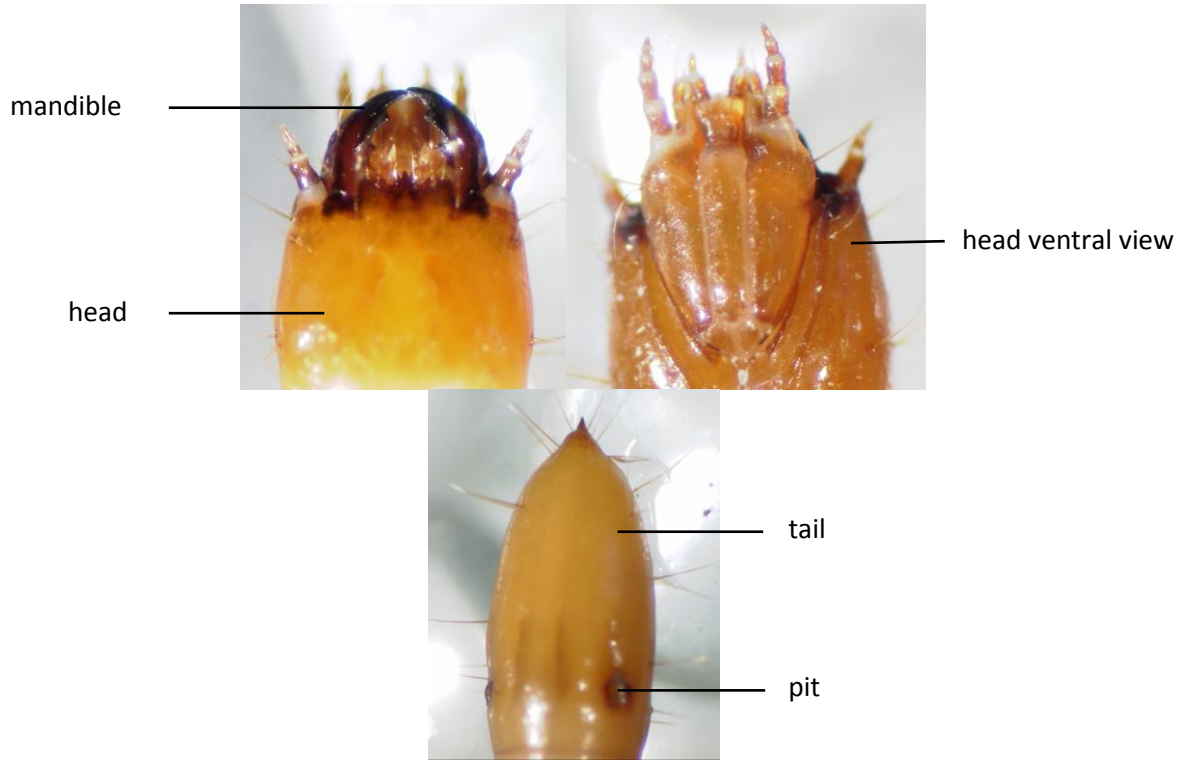

Figure 15: *Agriotes* larvae anatomy.

3.3.4 Damage assessment in potatoes

In early spring 2009, i.e., after five years of regular removal of all male click beetles, the grass-clover ley was inverted by ploughing. Hence certified virus-free pre-sprouted potatoes cv. *Belana* were planted on 15 April 2009 and managed according to the farm standards. Cultivar *Belana* is an early maturing variety, with a high number of medium tubers per plant. *Belana* has a stem-type foliage structure with very low flowering profusion. Tubers are oval shaped with deep yellow to slightly smooth netted skin colour. The buds drop readily and the intensity of anthocyanin colouration on the stem is moderate. Before the final harvest (potato harvester) from every plot two rows, each one measuring 5 m, were harvested by hand. From each row, i.e., two per plot, samples of 100 graded tubers (marketable size) were randomly selected, washed and assessed for wireworm damage using the following scale

Number of holes	0	1-2	3-5	>5
Scale	0	1	2	3

3.4 Effect of Naturalis (*Beauveria bassiana*) as a biological control for wireworms

The entomopathogenic fungus *B. bassiana* strain ATCC 74040 is widely regarded as one of the most promising species known for potential development in to a practical insect biological control agent (MCCOY 1990). Because of their distinctive mode of infection, fungi may play either a unique or a complementary role as insect biological control agents. Unlike bacterial, viral, or protozoan entomopathogens, fungi need not to be taken in by their hosts to cause an infection. Instead, germinating fungal spores can penetrate directly through the insect's cuticle and proliferate. It may take 3-5 days for insects to die. In the current study the effect of *B. bassiana* was assessed both in laboratory and field experiments.

3.4.1 Laboratory experiments

3.4.1.1 Wireworm collection and selection

Wireworms were collected by soil sampling and using bait traps from different infested untreated fields known to have a high *Agriotes* population. The species composition, determined was found to consist of two dominant species *A. lineatus* and *A. obscurus*, with an estimated prevalence of 60% and 40%, respectively. After collection the mixed population of wireworms was transferred to plastic vials filled with moist soil until required for tests (maximum 4 weeks) and periodically moisturized by water droplets to the water-holding capacity of soil. The wireworms selected for the experiment ranged from 4-13 instar stages, with active movement (a sign of good health). *Agriotes lineatus* and *A. obscurus* are regarded as pests to local crops, and both species are assumed to be treatable as a single group.

3.4.1.2 *Beauveria bassiana* seed coat application

Tests were done in plastic boxes (16.5 × 9.5 cm, depth 6.5 cm) containing a mixture of compost and sterilized sandy loam soil, a growing medium for the crop (Figure 16). The experiment contained three treatments (control and treated with *B. bassiana* application) with a different number of wireworms used per treatment. Each control treatment contained 2 wireworms per box, while in the other two treatments 3 per box (low number of wireworms) and 5 per box (high number of wireworms) were used. The number of applications per treatment was four. Wheat (*Triticum aestivum*) was used as a food source to draw wireworms to the seedlings. The boxes were sown with two rows at the rate of 15 seeds / row. To coat the wheat seeds with *B. bassiana* spores, seeds were first lightly dipped in canola oil to make the surface sticky, and then put in the spore solution for 10-15 minutes. Soon after the seeds were sown, wireworms (mixed accordingly instars) were released onto the surface of the soil at the centre of each box. Boxes were kept at 22°C and 50-90% relative humidity under fluorescent lights with 12h day length for 3-4 weeks. All the emerged plants were then counted and the

soils were sifted to recover the wireworms, which were then divided into two groups: alive and mobile or dead.

Figure 16: Treated and control boxes with wireworm population to check the effect of naturalis (*Beauveria bassiana*).

3.4.2 Field experiments

3.4.2.1 Experimental design and application of naturalis

The study was conducted from April to September in 2008 on a farm known to have a high wireworm (*A. lineatus*, *A. obscurus* and *A. sputator*) population. The area allotted for the experiment consisted of 12 completely randomized sub-plots, using three treatments: 1) untreated with no naturalis application, 2) sprayed on the ridges and 3) on the whole surface. Each sub-plot was replicated four times and had an area of 6 m x 6 m (36 m^2), while the entire plot area was 18 m x 24 m (432 m^2) used. For wireworm population assessment, each plot was sampled using a 13 cm diameter soil corer before planting the potato crop. No wireworms were found from whole samples, but this does not necessarily indicate an absence of wireworms in the field. Certified virus-free pre-sprouted seed tubers of cv. *Nicola* were planted on April 25 in 2008 within row spacing of 75 cm and within plants of 32 cm Naturalis (*B. bassiana*) was sprayed twice at the rate of 2.5 L/ha: at planting and after the emergence of plants at four weeks. At harvesting (September 8, 2008), 100 market-size potato tubers from each sub-plot were randomly selected to estimate the effect of the treatment by the presence of holes caused by wireworm feeding activity.

3.5 Statistical analysis

Data were evaluated through analysis of variance using SAS (version 9.1, SAS Institute, Cary, NC, USA). The percent recapture, in response to species, release distance, ground cover, and wind direction was evaluated by ANOVA using SAS. Normal distribution of the residues was checked through the SHAPIRO-WILK and KOLMOGOROV-SMIRNOV tests. For the post-hoc comparison of means we used the Tukey-Test ($\alpha \leq 0.05$). For more simple statistical evaluations t-Student test and standard deviation (SD) were calculated. For the range of attraction of sex pheromone traps the TURCHIN & ODENDAAL (1996) method was used. At first we evaluated the effect of species, wind direction and row distance in a three-factorial fully randomized design using the five trials as replications. Since no effect of wind direction on the recapture was noted, a second species-specific evaluation was carried out with ten replicates (5 trials * 2 wind directions). The objective was to identify and evaluate interactions between time and release distance and their impact on the beetle's recapture.

Based on the probability of beetle recapture P(r) from release distance r we calculated the maximum sampling range r_s and effective sampling area α of the traps according to TURCHIN & ODENDAAL (1996). The methodical approach is based on building regression models with combinations of untransformed and transformed data of recapture P(r) against release distance r. Calculations were done for two time periods, i.e., 12 and 30 days after release with *A. lineatus* and *A. obscurus*. The maximum sampling range (r_s) was calculated with the best fit regression by solving for P(r) = 0. For better comparison between both species we selected the relationship between log r to untransformed P(r), which showed the highest average R^2 over both species and time periods. The effective sampling area (α) was calculated using the equation proposed by ÖSTRAND & ANDERBRANT (2003) based on earlier work by TURCHIN & ODENDAAL (1996):

$$\alpha = 2\pi \int_0^{rs} rP\ r\ dr$$

Where:

r = release distance

r_s = maximum sampling range

P(r) = probability of recapture

α = maximum sampling area

The integrals were calculated using the online integrator WOLFRAM MATHEMATCIA (http://integrals.wolfram.com/index.jsp).

4 Results

4.1 Beetle collection and morphology

Adult click beetles (*A. lineatus*, *A. obscurus* and *A. sputator*) were collected in advance from a number of infested fields for the biological experiments in 2008. Adults of all three species were elongate, variable in size (Table 2) with the pronotum sharply pointed at the posterior corners and somewhat flattened in appearance (Figure 17). *Agriotes lineatus* and *A. sputator* adults showed a colour polymorphism within the same species. Body coloration of *A. lineatus* was yellow to brown while *A. sputator* was black-brown to reddish-brown. No colour dimorphism was observed within the same species of *A. obscurus* adults (see morphology details in Chapter 2 on page 9).

Table 2: Size of *A. lineatus*, *A. obscurus* and *A. sputator* male adults. Data obtained by measuring 300 specimens of each species collected at the experimental farm Wiesengut in 2008.

	Length (mm)				Width of abdomen close to prothorax (mm)			
	Mean	SD	Max	Min	Mean	SD	Max	Min
A. lineatus	9.04	0.52	10.65	7.84	2.70	0.19	3.21	2.15
A. obscurus	8.97	0.50	10.39	6.79	2.86	0.22	3.49	1.75
A. sputator	7.16	0.56	8.63	5.81	2.07	0.21	2.96	1.32

The females of all three species were slightly larger than males. The overall sex ratio of captured beetles was approximately 1:1; however; the ratios varied during the swarming period and in the beginning males were numerous than females, while in the middle of the swarming period females were significantly prevalent.

Figure 17: Adult male click beetles from left to right *A. lineatus*, *A. obscurus* and *A. sputator*.

4.2 Biology of *Agriotes lineatus* and *Agriotes obscurus*

This part of the thesis deals with the results of the field and laboratory experiments, which had been carried out to study the oviposition, larval development, pupation and adult activities of *A. lineatus* and *A. obscurus* in field conditions (rearing cages) and *A. obscurus* under controlled laboratory conditions. To develop strategies that best limit wireworm damage, a better understanding of the biology of both *Agriotes* species is required. The potential areas of weakness in the life cycle or behaviour of the insect can be exploited for controlling the pest.

4.2.1 Life cycle of *A. obscurus* under laboratory conditions

4.2.1.1 Eggs

4.2.1.1 Egg morphology and distribution

The collected beetles started oviposition a few days after introduction (April 2009) into plastic boxes filled with soil and fresh amended grass leaves. Eggs of *A. obscurus* were usually ovoid or oval but often irregular in shape and size (Table 3), presumably due to soil resistance against the ovipositor. The eggs were milky white to grey brown with a smooth surface (Figure 18). They remained the same color and shape until a few hours before hatching, at which time they collapsed during larval emergence. Eggs decreased in size and failed to develop when put in dry soil, presumably due to lack of moisture. Eggs were laid singly or in clusters.

Figure 18: ***Agriotes obscurus*** **eggs in the plastic boxes filled with moist soil.**

Egg clusters consisted of 2 to 39 individuals with batches laid close to one another. Most of the eggs were laid in the upper 2-3 cm of soil when the soil was moist. Some eggs were laid on the soil surface when the soil was covered by grass mulch.

Table 3: Average *A. obscurus* egg size measured on 150 eggs chosen randomly from different females, SD = standard deviation.

	Length (mm)	Width (mm)
Average	0.576	0.480
Maximum	0.755	0.572
Minimum	0.463	0.390
SD	0.044	0.040

4.2.1.1.1 Embryonic development and egg viability

The results of the experiment on the embryonic development of *A. obscurus* at 20°C showed that the eggs completed their development on average within 22.5 days (248 degree days, above a base of 9°C). Egg viability and hatching ranged from 95-100% based on more than 300 assessments. Newly hatched larvae were whitish in colour with a length of 1.89 to 2.57 mm (Figure 19).

Figure 19: Newly hatched *Agriotes obscurus* larva.

4.2.1.2 Larvae

4.2.1.2 Larval instars and development

Under laboratory conditions, between 8 to 11 instars were observed for *A. obscurus* (Table 4). The head size of the various instar stages showed a high degree of variability. It was often difficult to distinguish accurately between instar stages. First instar larvae were always recognized by the fact that the pits of the 9th abdominal segment could not be seen even under high microscopic magnification. The larvae reached a length of 11-16 mm at the 5th and 6th instar stages.

Table 4: ***Agriotes obscurus*** **larvae head size examined over 2 years (n = 85) fed on** ***Lolium multiflorum*** **L. grass in vials.**

Instars	Head width (mm)			
	Mean	SD	Min	Max
L1	0.20	0.016	0.17	0.23
L2	0.27	0.023	0.23	0.33
L3	0.34	0.039	0.27	0.46
L4	0.43	0.061	0.32	0.62
L5	0.55	0.078	0.39	0.78
L6	0.70	0.103	0.45	1.00
L7	0.87	0.151	0.58	1.34
L8	1.05	0.156	0.71	1.45
L9	1.26	0.158	0.91	1.53
L10	1.37	0.158	1.06	1.63
L11	1.46	0.129	1.26	1.67

There was an overlapping of the larval stages at each assessment date with the range of overlap being 0 to 4 instar stages. The overlapping range recorded over an 18 months period was 0 to 5 instar stages (Figure 20). The larvae stopped feeding before and after moulting due to body transformation. The larval body gradually enlarged in size by absorbing water with an appearance of white bands on either side of the body before moulting (Figure 22). At this stage the larvae slowed down their movement and formed a cell where the moulting has to occur. Under laboratory conditions with a constant temperature of 20°C, approximately 14% of the total population developed into adults while the remaining were still at larval stage (part of the larvae became dead). In the second year after egg-laying some of the *A. obscurus* larvae completed their life cycle in 14 months with only one overwintering and two calendar years, in other cases, most of the larvae entered into a second year of development with a second overwintering.

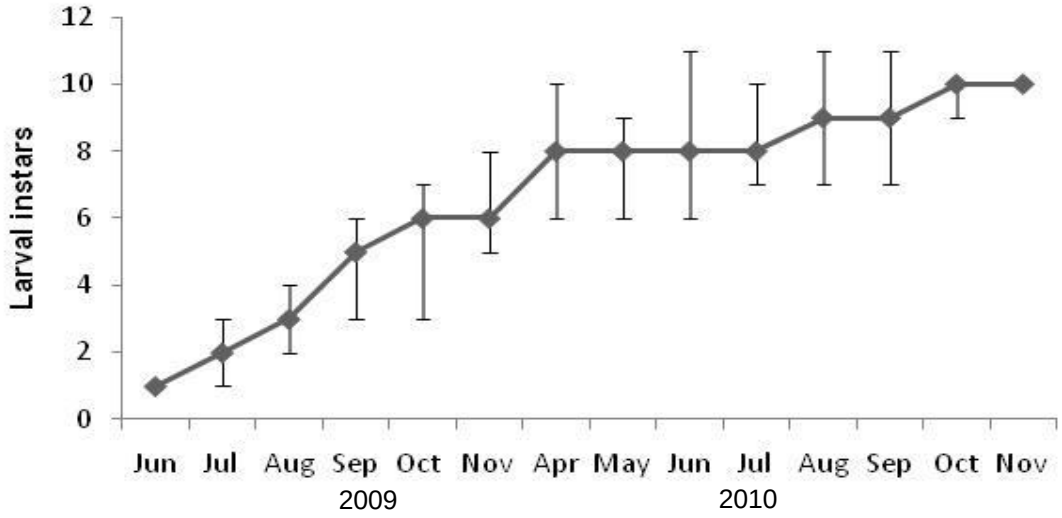

Figure 20: Development of larval instars from *A. obscurus* over an 18 months period at constant temperature (20°C in vials), I = minimum and maximum range values.

Larval development depended strongly on soil temperature even when adequate soil moisture and food were present. The temperature sum required to complete development increased with increasing larval age (Table 5). The final instar required about eight times more degree days (1,587 DD) to develop than needed for the first instar (190 DD). The average temperature sum required to complete development gradually increased from the first instar until the first overwintering stage (6^{th} and 7^{th} instar) with a slight decrease after overwintering till the ninth instar. There was again an increase for final instars (Table 6). The average total temperature required for all larval phases were about 9,248 degree days above a base of 9°C in the laboratory, corresponding to approximately 841 days. There was no difference noted in the day degrees needed for larval development of both sexes (between males and females).

Table 5: The average day degrees above a base of 9°C for the development of *A. obscurus* instar stages over 2 years in laboratory.

	Mean	SD	Max	Min
Eggs	248	25.48	275	187
1st instar larvae	190	14.92	198	165
Last instar larvae	1587	82.50	1628	1463
Pupae	169.5	23.67	220	143

4.2.1.2.1 Larvae survival

Wireworms feed on seeds and roots during their development. The mortality of younger, 2nd instars was 35% after 20 days of hatching and reached 48% in the 3rd and 4th instars. Approximately 56% mortality was observed before the first overwintering. The mortality rate decreased by 50% in the second compared to the first year. Resistance to starvation of newly hatched larvae was low. All the larvae died without food within 4-5 weeks after hatching. Cannibalism was observed between older and younger instars when food was scarce.

Table 6: ***Agriotes obscurus*** **larval instars (n = 85 larvae) development in days with average day degrees above a base of 9°C under laboratory conditions.**

	Days				
Instars	**Mean**	**SD**	**Max**	**Min**	**Heat sum > 9°C**
L1	17.3	1.36	18	15	190
L2	25.5	10.11	48	18	281
L3	46.9	15.73	89	33	516
L4	65.8	17.64	113	46	723
L5	83.3	16.82	109	45	917
L6	100.9	34.48	153	24	1110
L7	98.3	43.39	153	34	1082
L8	67.7	35.21	137	29	744
L9	83.5	31.65	120	37	919
L10	107.2	19.49	133	80	1179
L11	144.3	7.50	148	133	1587
Total	840.7		1221	494	9248

4.2.1.3 Overwintering in field soil

Larvae from the 4th to 8th instar had a survival rate of 100% when kept 60-80 cm deep in the soil during the winter from November to March. No diapause was observed and all the larvae started to feed and develop when returned to 20°C in the laboratory.

4.2.1.4 Pupae

Pupae were milky white in color and just before changing into adults the body transformed in to a light yellow color (Figure 21). In the final stages of pupal development the eyes darkened and became more visible.

Figure 21: Newly transformed *A. obscurus* pupa (Left) and adult (Right).

At 20°C the development from pupae to adults lasted an average of 15.4 days in vials. The average temperature sum required to complete pupal development was about 169.5 degree days above a base of 9°C (Table 7).

Table 7: Development rate and degree days above a base of 9°C for *A. obscurus* pupae to adults under laboratory conditions.

	Days	Degree days
Mean	15.4	169.5
SD	2.15	23.67
Max	20	220
Min	13	143

The adults darkened within 4-5 days at 20°C and the development into a hardened and perfectly coloured body took about 14-15 days. The sex ratio of newly emerged adults of *A. obscurus* was 72% males and 28% females.

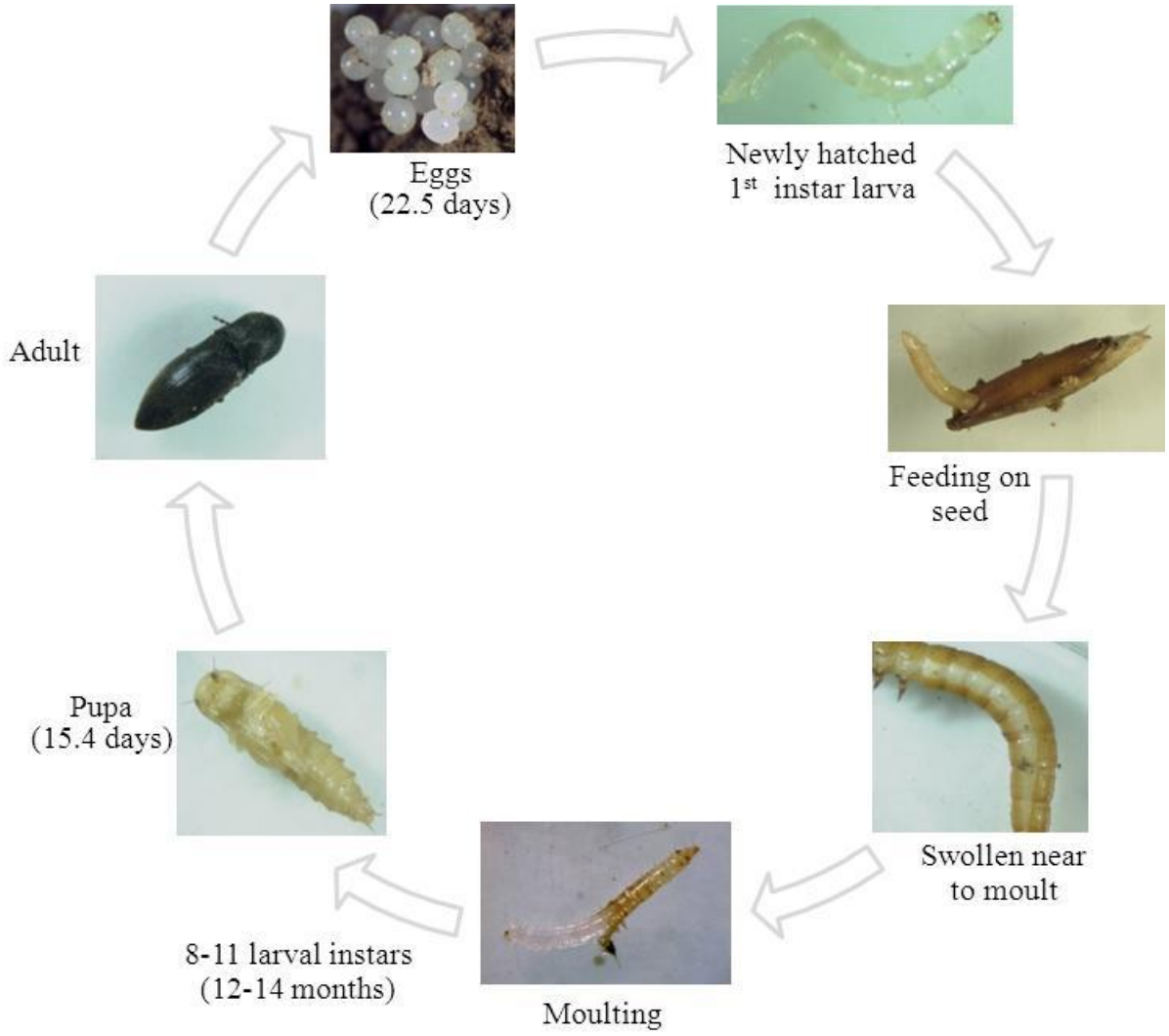

Figure 22: Life cycle of *A. obscurus* under laboratory conditions at constant temperature of 20°C.

4.2.2 Life cycle of *A. obscurus* and *A. lineatus* in semi-natural conditions in rearing cages

4.2.2.1 Eggs

The first eggs of *A. obscurus* and *A. lineatus* were observed in late April. Peak oviposition occurred between May and early June. It took 15-20 days after the introduction of male and female till egg-laying initiation (see details of egg morphology and distribution on page 47).

4.2.2.2 Larvae development

In total 13 larval instars for *A. obscurus* and 12 for *A. lineatus* were observed after 3 years (Figure 23). The majority of the larvae reached the 3rd to 6th instar stage and measured 8 mm of length before their first overwintering. Larval development again began in May of the following year for both species. Larval stages overlapped at each assessment date with the range of overlap being 0 to 5 instar stages for *A. obscurus* and *A. lineatus*. The overlapping range recorded over a thirty months period was 0 to 7 instar stages for *A. obscurus* and 0 to 6 for *A. lineatus*. The highest number of instars for both species (13 for *A. obscurus* and 12 for *A. lineatus*) was observed in the month of October during the second year of development. Larvae from the 7th to 12th instar stage entered into a second overwintering and exceeded 18 mm length for both species.

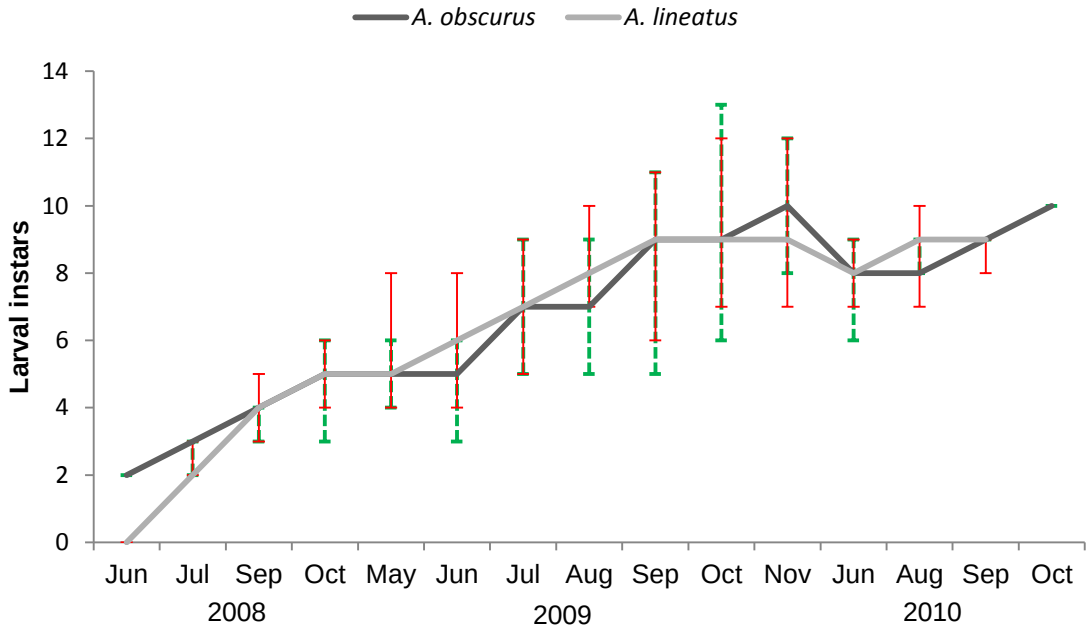

Figure 23: Development of larval instars from *A. obscurus* and *A. lineatus* over a 30 months period in rearing cages in the field, I = minimum and maximum range values.

4.2.2.3 Larval population density in rearing cages

The maximum larval population was captured in the second year of development for both species (Table 8 & 9). The highest number of *A. obscurus* was recorded in June 2009 when 111 larvae were captured followed by 44 specimens in August of the same year (Table 8). *Agriotes lineatus* was captured at high densities three months later than *A. obscurus* in October 2009 when 91 larvae were recorded, followed by 43 specimens in November of the same year (Table 9). The majority of the larvae during the second year were in the 5^{th} to 9^{th} instar stages, considered the most damaging stage during the insect's life cycle (FURLAN 2004). There were very low number of larvae trapped by soil sampling and bait traps during the first and third year of development in rearing cages. Of the total captures in three years most of the larvae (90% of *A. lineatus* and 83% of *A. obscurus*) attracted to bait traps in the second year were considered to be at the most mobile and effective period of their life cycle.

Table 8: ***Agriotes obscurus*** **larval development in rearing cage cropped with corn, wheat, rye, mustard and grass-clover over the period of 2008-2010. Data are sum of two bait traps in one cage; larval population estimated by soil sampling and bait traps.**

	N° of trapped Larvae/ cage	Percentage of total larvae per instar												
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
June 2008	5		**100**											
July	7		29	**71**										
August	0													
September	6			33	**67**									
October	20			20	5	**70**	5							
May 2009	39				10	**67**	23							
June	111			5	22	**58**	15							
July	35					11	26	**49**	11	3				
August	44					7	18	30	**43**	2				
September	24					4	4	8	17	**46**	4	17		
October	24						4		17	**42**	21	8	4	4
November	7								15	**57**		14	14	
May 2010	0													
June	10						10	**40**	30	20				
July	0													
August	5								**60**	40				
September	2									**100**				
October	2										**100**			

The variability among the larval length of the same instars and the diversity of instars was noticeable at a single assessment date.

Table 9: ***Agriotes lineatus*** **larval development in rearing cage cropped with corn, wheat, rye, mustard and grass-clover over the period of 2008-2010. Data are sum of two bait traps in one cage; larval population estimated by soil sampling and bait traps.**

	N° of trapped Larvae/ cage	Percentage of total larvae per instar												
		1	2	3	4	5	6	7	8	9	10	11	12	13
June 2008	0													
July	4		**75**	25										
August	0													
September	5			20	**60**	20								
October	6				33	17	**50**							
May 2009	14				21	**36**	**36**		7					
June	28				4	21	18	**43**	14					
July	14					7	29	14	**36**	14				
August	31							13	**45**	36	6			
September	35						3	14	23	**43**	9	8		
October	91							2	24	**33**	21	11	9	
November	43							2	30	**49**	9	5	5	
May 2010	0													
June	6							33	17	**50**				
July	0													
August	6							16	17	**50**	17			
September	4								25	**75**				
October	0													

4.2.2.4 *Agriotes obscurus* development in the laboratory and field

Agriotes obscurus showed a significant difference in the rate of larval development under laboratory and field conditions (Figure 24). The average number of instars observed in the field was 4.7 compared to 6.2 in the laboratory after the first year of development. The larvae reached a maximum 11th instar stage after 14 months of egg laying in the laboratory compared to field conditions where they reached a maximum of 13 instar stages 18 months after egg laying. Larval peak instar stages were recorded during the second year of development in both rearing conditions, with some of the larvae transformed into adults in the laboratory completing their life cycle in 14 months after egg laying.

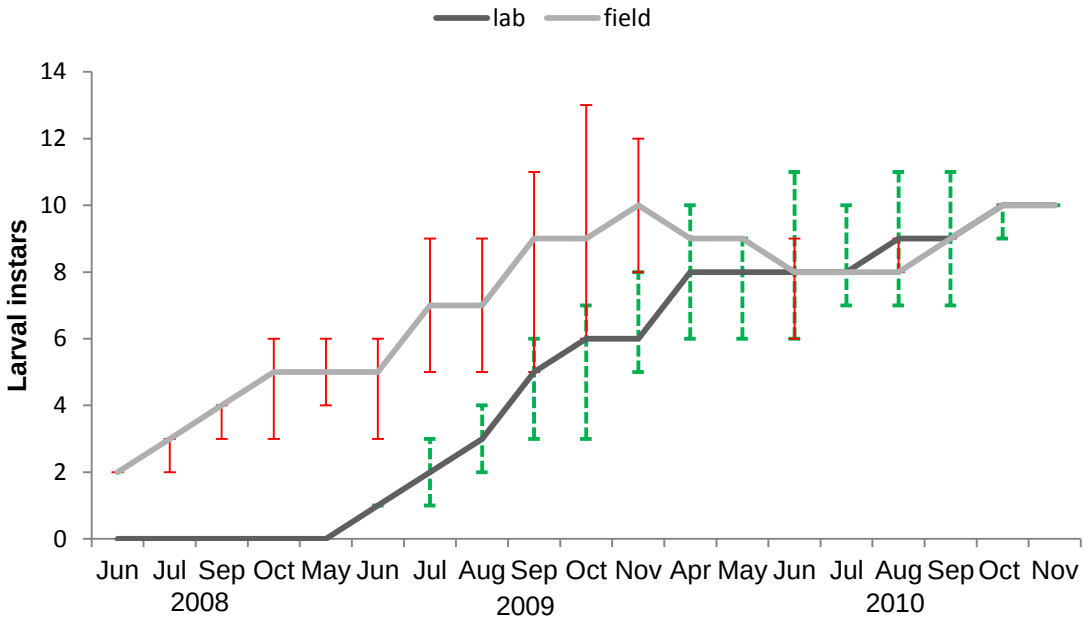

Figure 24: Development of larval instars from *A. obscurus* over an 18 months period in the laboratory and 30 months period in the rearing cages in the field, I = minimum and maximum range values.

4.3 Range of attraction of pheromone traps (Mark-release-recapture)

This part of the research work deals with results of three years of experimentation to assess the effectiveness of *A. lineatus* and *A. obscurus* sex pheromone traps by using two types of soil coverage (grass clover and bare soil) during natural dispersal peaks (May and June) of the beetles from 2006-2008. Pheromone assessment helps to determine the distribution and spread of wireworm infestations.

4.3.1 Total recapture of *A. lineatus* and *A. obscurus* males

A total of 3,120 males of *A. lineatus* and *A. obscurus* were released and, in total, 1,257 (40.3%) of the marked males were recaptured over all five trials. Males were recaptured from all 12 release points in both wind directions. Averaged over both species, the lowest percentage recapture was noted in trial T2 (32%) and the highest in trial T3 (47%). The range of recaptured beetles over all trials was comparable for both *A. lineatus* (32-53%) and *A. obscurus* (30-59%) (Table 10).

Table 10: Percentage recapture of *A. lineatus* and *A. obscurus* male beetles released at different distances from pheromone traps assessed over a 4-week period in five trials during 2006-2008.

	% Recapture														
	2 m		**5 m**		**10 m**		**15 m**		**20 m**		**60m**		**Total[a]**		
Trial	lin	obs	lin	obs	lin	obs	lin	obs	lin	obs	lin	obs	lin	obs	(%)[b]
1 (600)[c] [2006-1]	94	94	74	68	56	34	30	22	26	12	2	6	**141**	**118**	**43**
2 (600) [2006-2]	62	60	54	42	50	28	18	26	16	18	6	0	**103**	**89**	**32**
3 (600) [2007-3]	90	46	66	58	52	42	54	42	32	38	22	18	**158**	**122**	**47**
4 (720) [2007-4]	87	87	30	67	17	60	25	77	13	53	20	10	**115**	**212**	**45**
5 (600) [2008-5]	76	58	46	52	40	36	22	24	26	18	0	0	**105**	**94**	**33**
Mean	82	70	53	58	42	42	30	40	22	29	10	7	**124**	**127**	**40**

[a]Total recaptured beetles from all distances.

[b]Average percentage recapture per trial

[c]Trial number (number of released beetles per trial) [experimental year]. *lin, Agriotes lineatus*; *obs, Agriotes obscurus*

A three-factorial analysis showed no significant interactions between the factors species, release distance and wind direction. Species and wind direction had no impact on the overall percentage of recaptured beetles (Figure 25). Averaged over both species and wind directions, a clear decrease of the recaptures was noted with increasing release distance. The percentage recapture at 2 m release distance was significantly higher (75%) compared with all other release distances. Recaptures at the release distances 5, 15, 20 and 60 m were also significantly different to each other. The highest level of recapture was for *A. lineatus* (42%) compared to *A. obscurus* (35%) in four out of five trials, except trial T4 when *A. obscurus* recovery was almost double that of *A. lineatus*. The decrease in the percentage of recaptured beetles with increasing release distance followed a similar pattern for both species. The experiment clearly demonstrated that the recapture of released beetles was mainly dependent on release distances than on time.

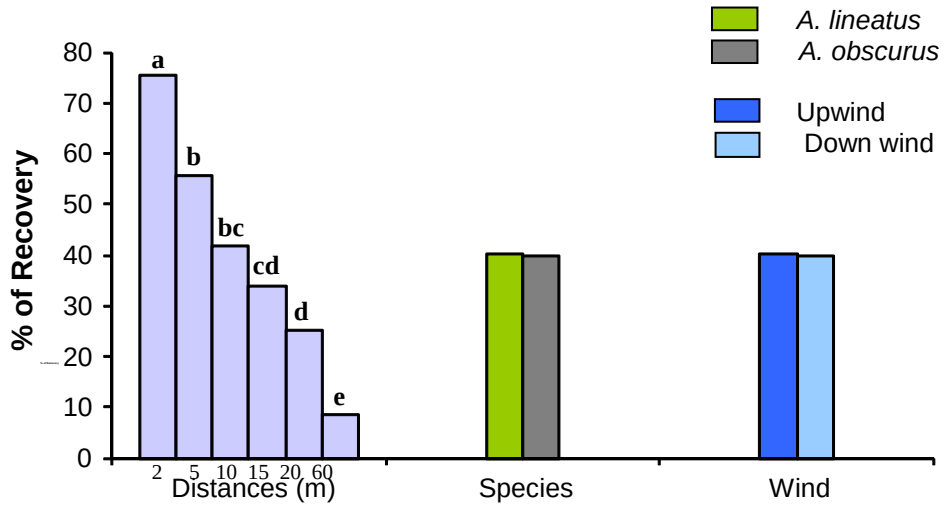

Figure 25: Effect of release distance, species and wind direction on percent recapture of male click beetles (*A. lineatus* and *A. obscurus*) in pheromone traps. Means with the same letters are not significantly different at α = 0.05 (Tukey-test).

4.3.2 Recapture of *A. lineatus*

From a total of 1,560 *A. lineatus* males released, 622 beetles were recaptured over a 4-week period in five trials during 2006-2008. Recapture varied from 53% (trial 3) to 32% (trial 4). The maximum level of recapture was 94% at a release distance of 2 m with a significant decrease in recapture over longer distances. The maximum recapture at a release distance of 60 m was 22% noted in trial T3 in contrast to trial T5, when no beetles were recaptured from the same distance. After approximately one month of experimentation 82% of the *A. lineatus*

beetles released at a distance of 2 m from the trap were recaptured, compared to 53% of 5 m, 42% at 10 m, 22% at 20 m and 10% at 60 m (Table 10).

Most *A. lineatus* males were recaptured within 3 days after release independent of the distance except for 60 m (Figure 26). The proportion of late-comers tended to be higher for beetles released at larger distances for both species. The peak of recapture for *A. lineatus* was recorded after one day for a release distance of 2 m. The rate of recapture significantly decreased over 3 and 5 days after release for all distances up to 10 m. The peak level of *A. lineatus* recapture was also observed after one day at 2 m release distance when recovery was 47.2% (Figure 26). The recapture pattern of twelve days and more after release was comparable for both species and never exceeded 8.5% recapture.

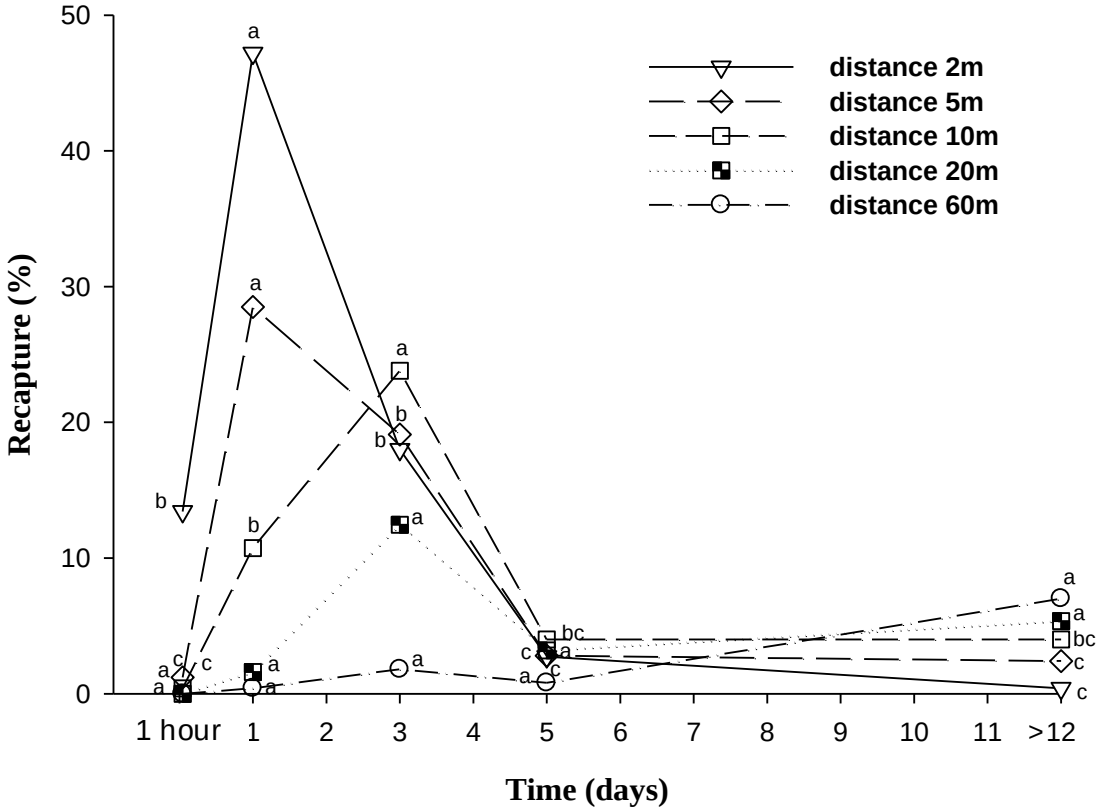

Figure 26: Percent recapture of *A. lineatus* in pheromone traps as affected by different time periods and release distances. Average of five trials over three years (2006-2008). Means with the same lowercase letters are not significantly different at α = 0.05 (Tukey-test).

4.3.3 Recapture of *A. obscurus*

From a total of 1,560 *A. obscurus* released, 635 beetles were recaptured over a 4-week period in five trials during 2006-2008. The highest level of recapture was 59% (trial 4) compared to a low of 30% (trial 2) specimens. The maximum level of recapture was 94%, similar to *A. lineatus* at a release distance of 2 m with a significant decrease over longer distances. The maximum recapture at a release distance of 60 m was 18% compared to a low of zero beetles recaptured from 60 m release distance. At the end of the experiments 70% of the *A. obscurus* beetles released at a distance of 2 m from the trap were recaptured, compared to 58% at 5 m, 42% at 10 m, 29% at 20 m and 7% at 60 m respectively (Table 10).

Most *A. obscurus* males were recaptured within 3 days after release independent of the distance except at 60 m (Table 11). After 1 hour, recapture was only noted at 2 and 5 m distances compared to 1 and 3 days at all other release distances except for 60 m. The peak ratio of recapture for *A. obscurus* beetles was recorded after one day at a release distance of 2 m with 29.76% specimens. Recapture significantly decreased over increasing distances for all time periods. At twelve days and above no significant difference was noted in recapture beetles for all released distances (Table 11). After five days up from release distances of 20 m the recapture of *A. obscurus* was significantly lower compared to the shorter distances.

Table 11: Percent recapture of *A. obscurus* in pheromone traps in different time periods at different release distances in all five trials over the years of 2006-2008. Means with the same lowercase letters are not significantly different at $\alpha = 0.05$ (Tukey-test).

	Distance (m)					
Time (days)	**2**	**5**	**10**	**15**	**20**	**60**
1hr	12.06 **a**	2.40 **b**	0.00 **c**	0.00 **c**	0.00 **c**	0.00 **c**
1	29.76 **a**	19.10 **b**	8.10 **c**	6.40 **cd**	3.33 **cd**	0.00 **d**
3	12.13 **a**	19.23 **a**	20.30 **a**	15.36 **a**	11.50 **a**	0.00 **b**
5	9.06 **a**	10.40 **a**	7.60 **a**	9.13 **a**	4.50 **ab**	1.10 **b**
>12	5.86 **a**	6.26 **a**	4.80 **a**	7.20 **a**	8.46 **a**	5.70 **a**

4.3.4 Maximum sampling range and effective sampling area

The probability of recapture from a specific release distance is important information for trap spacing. A more accurate approach may be given by calculating the maximum sampling range and the effective sampling area of the traps. The maximum sampling range indicates the distance from which an insect can reach the trap within a given time period. The effective sampling area indicates the theoretical area around the pheromone trap from which all beetles will be trapped. The maximum sampling range and the effective sampling area were calculated for *A. lineatus* and *A. obscurus* using the Log r to recapture relationship (Table 12). The maximum sampling range r_s for *A. lineatus* twelve days after release was 54.7 m and increased to 72.2 m after 30 d. The corresponding values for *A. obscurus* were 71.7 m for 12 d and 95.5 m for 30 d and were considerably higher. The effective sampling area for *A. lineatus* was 1,089 m² after twelve days and increased to 1,735 m² after 30 days. The maximum sampling range of the effective sampling area for *A. obscurus* was higher when compared with *A. lineatus* of 1,518 m² for 12 d and 2,633 m² for 30 d respectively.

Table 12: Calculated regressions for the relationship between release distance (r) and the probability of recapture P(r) and estimated sampling ranges (rs) and areas (α) for two click beetle species after two time periods.

		r	Log r	r	Log r	Sampling range (r_s)	Sampling area (α)
	Number of days	*P(r)*	*P(r)*	Log *P(r)*	Log *P(r)*	(m)	(m²)
A. lineatus	12	y = -0.01x + 0.56 R^2 = 0.47	y = -0.53x + 0.92 R^2 = 0.72	y = -0.02x - 0.21 R^2 = 0.68	y = -0.70x + 0.19 R^2 = 0.74	**54.69**	**1089**
	30	y = -0.0094x + 0.58 R^2 = 0.42	y = -0.49x + 0.91 R^2 = 0.66	y = -0.01x - 0.23 R^2 =0.49	y = -0.62x + 0.16 R^2 = 0.60	**72.19**	**1735**
A. obscurus	12	y = -0.0094x + 0.53 R^2 = 0.47	y = -0.44x + 0.81 R^2 = 0.57	y = -0.02x - 0.24 R^2 = 0.53	y = -0.58x + 0.06 R^2 = 0.50	**71.70**	**1518**
	30	y = -0.0093x + 0.57 R^2 = 0.50	y = -0.43x + 0.84 R^2 = 0.60	y = -0.01x - 0.23 R^2 = 0.55	y = -0.56x + 0.08 R^2 = 0.54	**95.46**	**2633**

4.4 Prevention trial (Mass trapping)

This part of the thesis deals with the long-term field experiments from 2004-2009 to assess species numerical fluctuation and regular male mass trapping of three key *Agriotes* species over five years to decrease wireworm abundance and subsequent damage in potatoes.

4.4.1 Male trapping

A total of 12,378 male adults of *A. lineatus*, *A. obscurus* and *A. sputator* were captured by using YATLOR sex pheromone traps (Figure 27). On average 2,063 beetles were caught per trap from both treated plots during the course of the experiment from 2004-2008. The highest rate of capture was in 2007 when 3,075 beetles (*A. lineatus*, *A. obscurus* and *A. sputator*) were captured, followed by 2008 when 2,986 specimens were trapped. The lowest level of trapping was in 2005 when 1743 males were captured compared to the first trapping year (2004) with 2,372 specimens. There was a slight decrease in population noted in the second year followed by a gradual increase in beetle trapping until the last year.

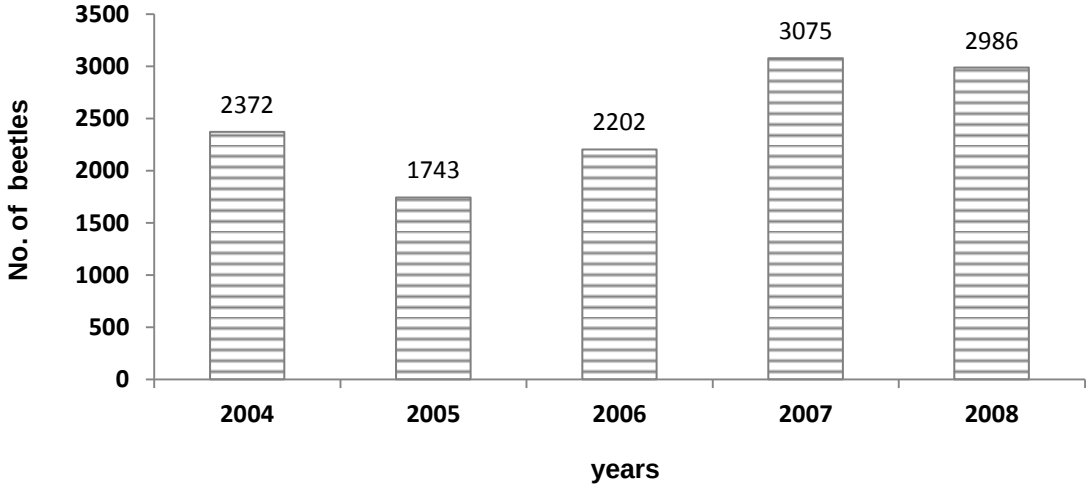

Figure 27: Total number of *Agriotes* species captured in pheromone traps over the period 2004-2008.

Trapped beetles were classified to low, medium and high levels of capture (Table 13). The classification of population density within Europe is as follows (FURLAN et al. 2001b): High population level = more than 500 adults/trap/season; Medium population level = between 50 and 500 adults/trap/season and Low population level = less than 50 adults/trap/season. During

the first two years in 2004 and 2005 beetles were captured at a medium population level. *Agriotes lineatus* was captured at high densities in 2006 and 2007 followed by medium level in 2008, while *A. obscurus* was trapped at a medium population level in 2006-07 and at high population level in 2008. Similarly, *A. sputator* was also trapped in low and medium population levels.

Table 13: Adult population level estimated by capturing *Agriotes* beetles using sex pheromone traps during 2004-2008.

Year	*Agriotes* species	*Agriotes lineatus*	*Agriots obscurus*	*Agriotes sputator*
2004	Medium	-	-	-
2005	Medium	-	-	-
2006	-	High	Medium	Low
2007	-	High	Medium	Medium
2008	-	Medium	High	Medium

Species classification was started in 2006 and *A. lineatus* was shown to be dominant in that year with 1,236 specimens, followed by *A. obscurus* (892) and a surprisingly low level of *A. sputator* (74). In 2007 again the most trapped species was *A. lineatus* (2,161) compared to *A. obscurus* (549) and *A. sputator* (365) specimens. *Agriotes obscurus* was recorded in high numbers (1,604) in 2008 in contrast to *A. lineatus* (608) and *A. sputator* (774). The dominant species captured with the pheromone traps were *A. lineatus* and *A. obscurus* with a share of 49% and 37% of total captured beetles, respectively. In contrast, total pheromone trap captures of *A. sputator* from 2006 to 2008 were considerably lower with only 14% of all captured beetles (Table 14).

Table 14: Percentages of total male (*A. lineatus*, *A. obscurus* and *A. sputator*) captures in six pheromone traps (2 per species) during 2006-2008.

	2006	2007	2008
A. lineatus	56	70	20
A. obscurus	41	18	54
A. sputator	3	12	26

Conspicuous yearly differences were noted in flight dynamics among all three species during 2006-2008 (Figure 28-32). The swarming period lasted from late April to late August with one major peak and a minor peak in the successive years. In 2004 adult peak flights were observed in the first and second week of June and at the end of July when 37 and 36 males were captured per day respectively (Figure 28). There was a sharp decline after the first peak when only five adults were captured per day in the third week of June followed by an erratic increase until the second peak.

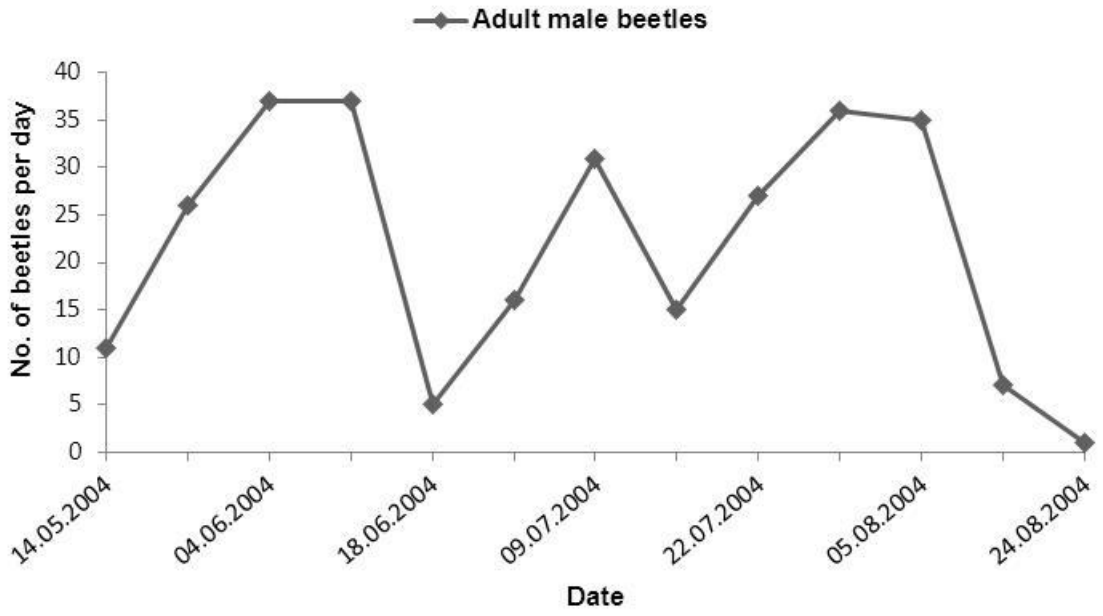

Figure 28: Adult males of *A. lineatus*, *A. obscurus* and *A. sputator* (number of beetles per day) captured in six pheromone traps during 2004.

Compared with 2004 the first peak was about six weeks earlier in 2005 and the second smaller peak in the third week of June, followed by a steady decrease until the end of August (Figure 29). The first peak was directly after trap installation with the capturing of 116 males per day and dropped to 23 males during the next week. In 2006 the adult active period lasted from the beginning of May to the middle of August with one main peak in mid May and one smaller peak in the middle of June for both *A. lineatus* and *A. obscurus*, respectively (Figure 30). Of the total capture of *A. lineatus* and *A. obscurus* together more than 50% males were trapped during the second week of May in 2006. There was a distinct decrease in captures of both species after the first peak flight up until the middle of June when a second peak was recorded with 36 and 11 males per day of *A. lineatus* and *A. obscurus*, respectively.

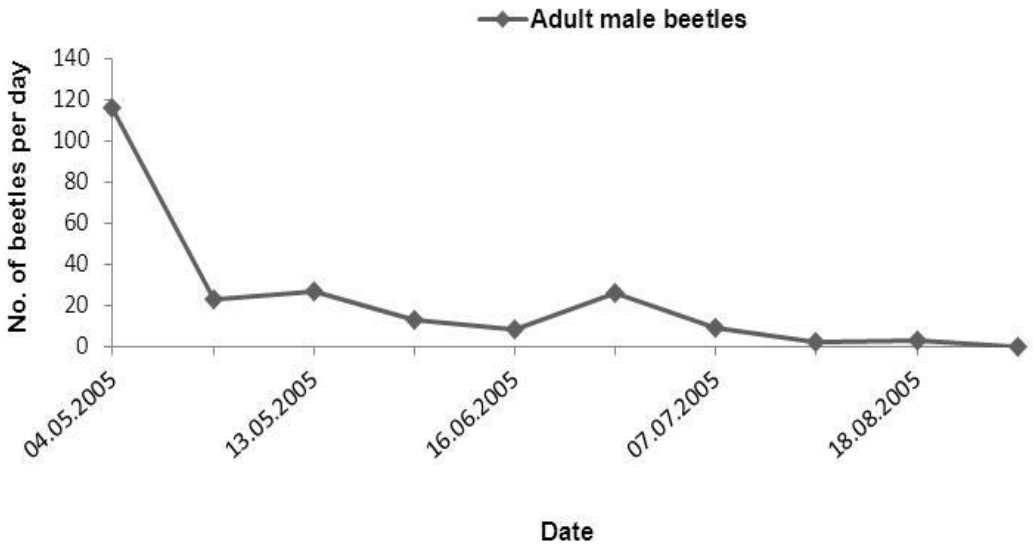

Figure 29: Adult males of *A. lineatus*, *A. obscurus* and *A. sputator* (number of beetles per day) captured in six pheromone traps during 2005.

The flight pattern in 2007 recorded one major peak at the end April with a second peak nearly one month later at the beginning of June (Figure 31). Of the total capture of *A. lineatus* and *A. obscurus* together more than 42% males were trapped during the last week of April in 2007. The flight terminated much more abruptly after the second peak with very few captures from mid-June until the end of the swarming period. A similar flight pattern with two peaks in May and June and relatively more uniform captures throughout the swarming period were observed in 2008 (Figure 32). Comparing the trap catches in both plots, no significant difference was noted among individual traps for all three species. Averaged over five years (2004-2008) about 67% of the total males of the three species were caught in the period from the last week of April till the beginning of June. The lowest capturing period was the month of August when only 4% of the total *A. lineatus*, *A. obscurus* and *A. sputator* were trapped. There was no evidence of any decisive effect of temperature on the size of trap catches, though some minor impacts of sum of temperatures were noted on flight peaks of *Agriotes* species (Table 15). In 2006 the first flight peak of *A. lineatus* and *A. obscurus* was at a sum of 691°C temperature, in contrast to *A. sputator* at 1,861°C temperature. A similar temperature effect on flight pattern was noted in 2007 followed by no significant difference between the three species in 2008.

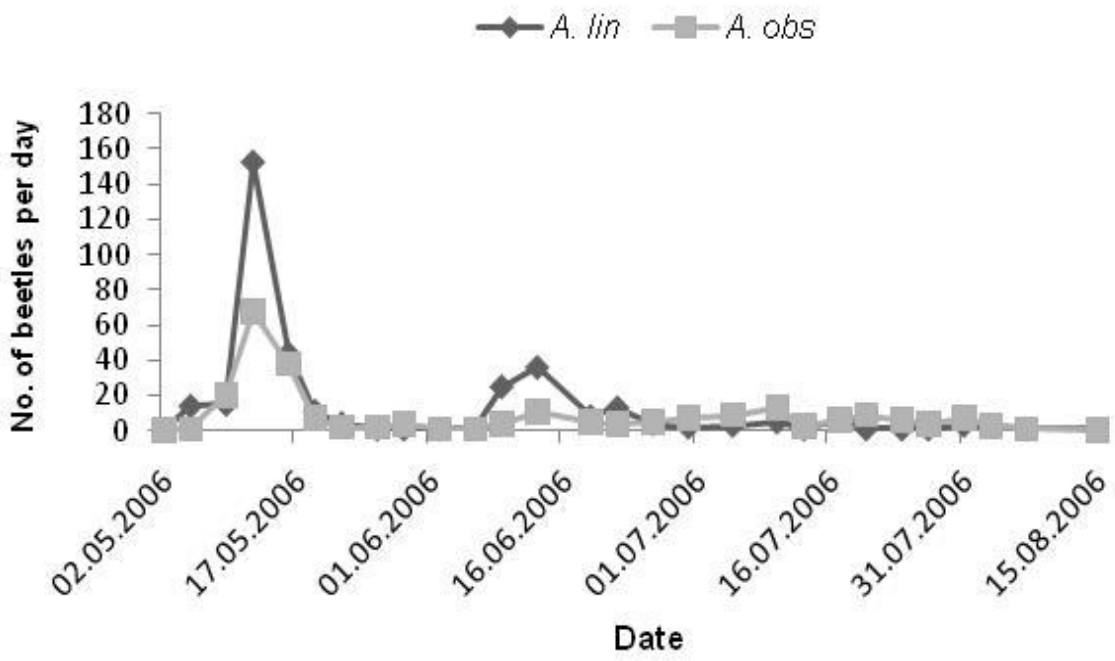

Figure 30: Adult males of *A. lineatus* and *A. obscurus* (number of beetles per day) captured in four pheromone traps during 2006.

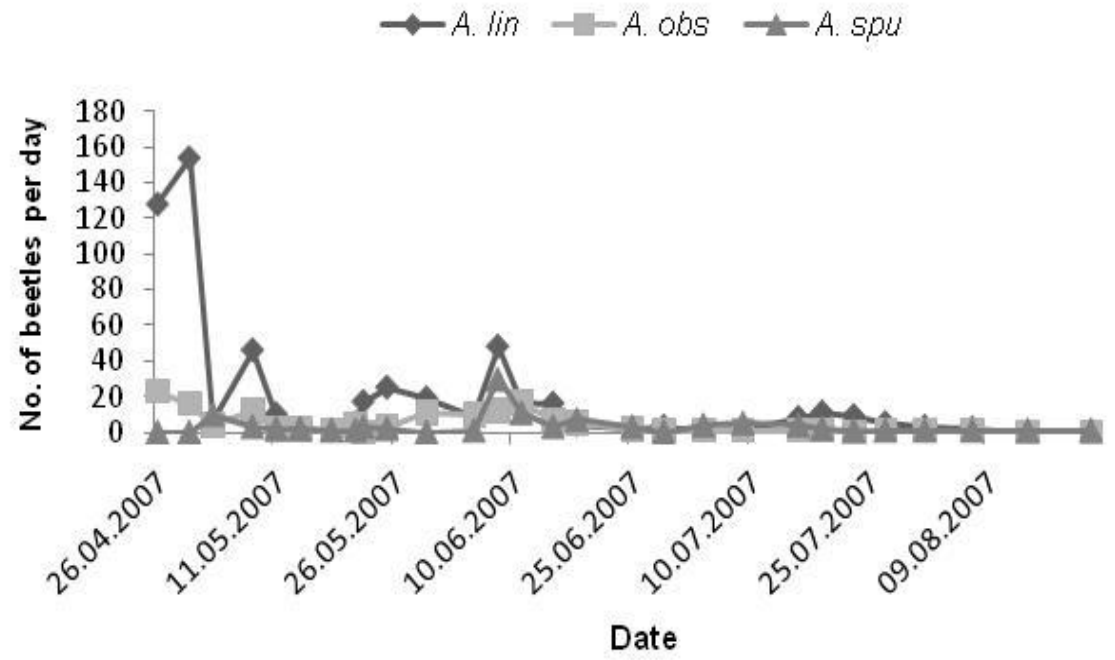

Figure 31: Adult males of *A. lineatus*, *A. obscurus* and *A. sputator* (number of beetles per day) captured in six pheromone traps during 2007.

Beetles have a concealed mode of life and are seen when the soil heats up to 10-15°C. In our study adults become active during the 2nd half of the day and observed flights coincided with warm days, at a temperature of approximately 20-25°C under sunny skies.

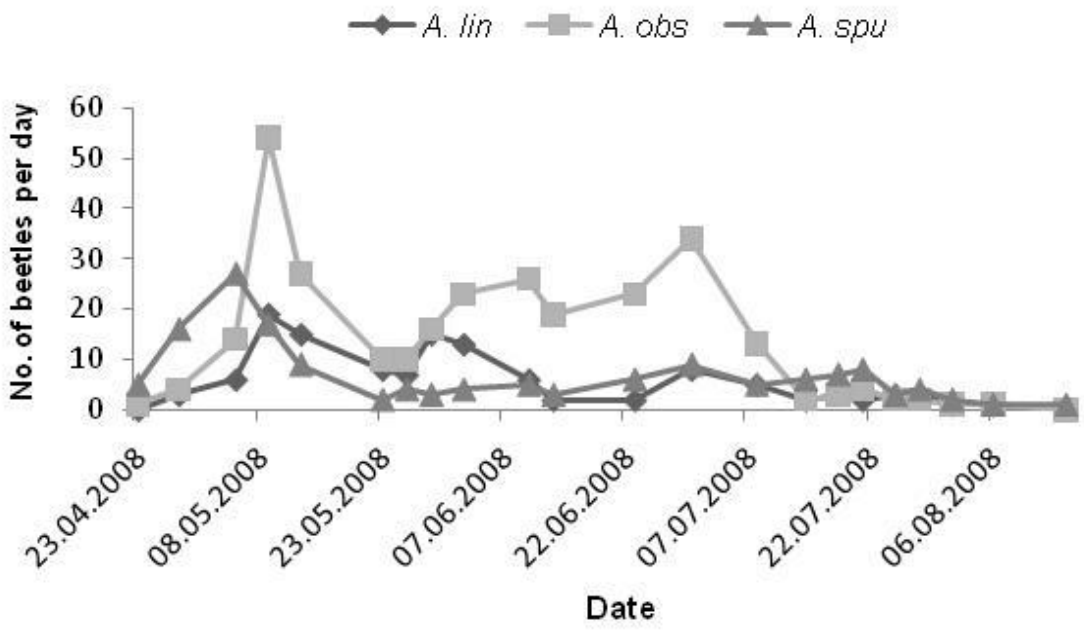

Figure 32: Adult males of *A. lineatus*, *A. obscurus* and *A. sputator* (number of beetles per day) captured in six pheromone traps during 2008.

Pheromone traps proved to be highly selective in distinguishing between the species, with sporadic catches of both *A. lineatus* and *A. obscurus* in one specific trap due to the presence of the common pheromone component (Geranyl octanoate). Untargeted species (spiders, earwigs and carabidae) trapped were less than 1%.

Table 15: Sum of air temperatures above 0°C from the beginning of year to the first flight peak of three *Agriotes* species (*A. lineatus*, *A. obscurus* and *A. sputator*) during 2006-2008.

	Σ of υ in °C from the beginning of year to first flight peak		
Species	**2006**	**2007**	**2008**
A. lineatus	691	1024	905
A. obscurus	691	952	905
A. sputator	1861	1665	838

4.4.2 Wireworm population

Overall 431 wireworms, exclusively *Agriotes* species, were captured during the sampling seasons from 2004-2009. Wireworms were obtained in every sampling period throughout the season from both treated and untreated plots. Of all the wireworms identified during the study period, *A. obscurus* was the most abundant (165 individuals), followed by *A. lineatus* (162 individuals) and *A. sputator* (15 individuals), except 2004 when larvae were not identified at species level. Other species found could not be identified accurately.

There was no conspicuous difference in capture percentages of *A. lineatus* (43-59) and *A. obscurus* (39-60) over the period from 2006 to 2009 (Table 16). In contrast *A. sputator* showed great variation compared to *A. lineatus* and *A. obscurus* with 0 to 15% being captured. In 2006 the most trapped species was *A. obscurus* (60%) followed by *A. lineatus* (40%) specimens. Comparable patterns were observed in 2007; confoundingly no *A. sputator* was captured in 2006-07. During the last two years *A. obscurus* and *A. lineatus* were also the most abundant captured species with a small share of *A. sputator* (about 15% each year).

Table 16: Percentages of total wireworms (*A. lineatus*, *A. obscurus* and *A. sputator*) captures by soil sampling during 2006-2008.

	2006	2007	2008	2009
A. lineatus	40	59	46	43
A. obscurus	60	41	39	43
A. sputator	0	0	15	14

In contrast to our expectations of the total amount of wireworms captured over all sampling dates, there was only a slight non-significant difference between pheromone-treated (201 individuals) and control plots (230 individuals). Three months after the beginning of the experiment (August 2004) the total number of wireworms captured with the soil sampling method was 0.58 wireworms per soil core in the treated and 0.31 in the untreated plots. However, at two out of nine sampling dates, the number of wireworms per soil score was significantly lowers in pheromone-treated as compared with control plots. The highest difference was noted in October 2006 with 0.26 compared with 0.88 wireworms per soil core (Figure 33).

Two years later (no soil sampling was done in 2005) in June 2006 the total number of wireworms detected tended to be higher in untreated compared with treated plots. The number of wireworms of the trapped beetle species *A. lineatus*, *A. obscurus* and *A. sputator* was

significantly lower in the treated compared with 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*, *A. obscurus* and *A. sputator* between cleaned plots (0.13 wireworms per soil core) and control plots (0.28 per soil core) were not significant. One year later in 2007 a slight difference per soil core was observed in wireworm populations in both plots, suggesting a gradual decrease of population after repeated male trapping over the years. Similar findings were noted in May 2008, but interestingly there was a high population in treated plots compared with the untreated plots in two later samplings.

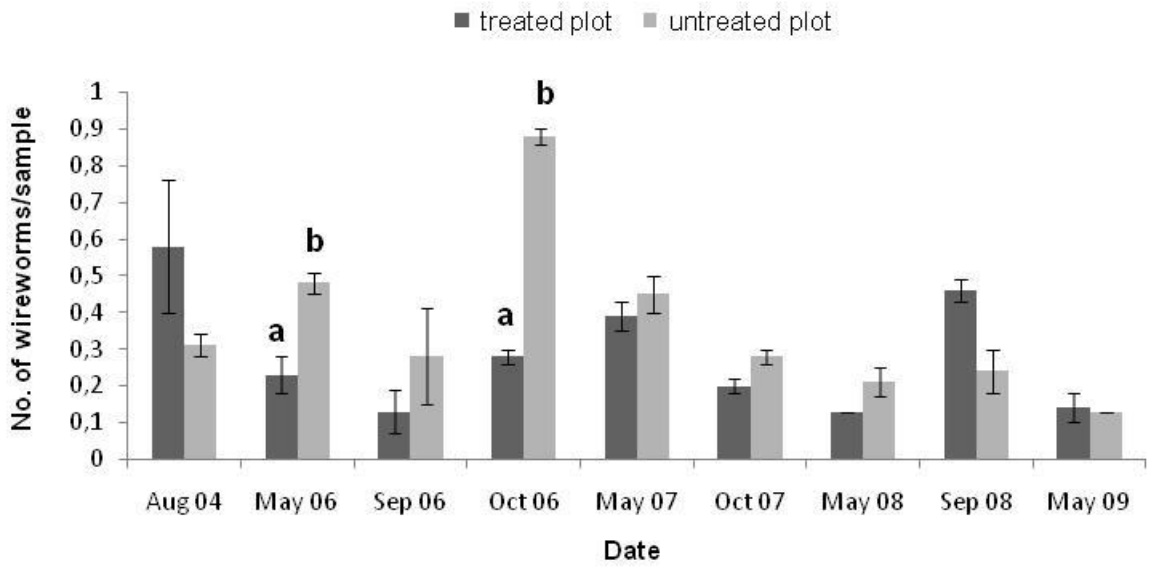

Figure 33: Number of wireworms (*A. lineatus, A. obscurus* and *A. sputator*) detected per soil core (±SE) from 2004-2009 Tukey's test (α = 0.05).

In our study the wireworms captured ranged from 2nd-13th instar stages with ubiquitous field distribution and the presence of overlapping generations of all three species in the treated and untreated plots. The development of wireworm abundance over time did not follow any consistent pattern. The larval population of *Agriotes* species was widespread across the plots with some patchy distribution. There was no clear difference demonstrated on the effectiveness of pheromone traps among the treated and untreated plots (Figure 34). Another intriguing finding was the capturing of wireworms within 3-5 meters radius of the sex pheromone traps during the last three years of soil sampling (2007-2009).

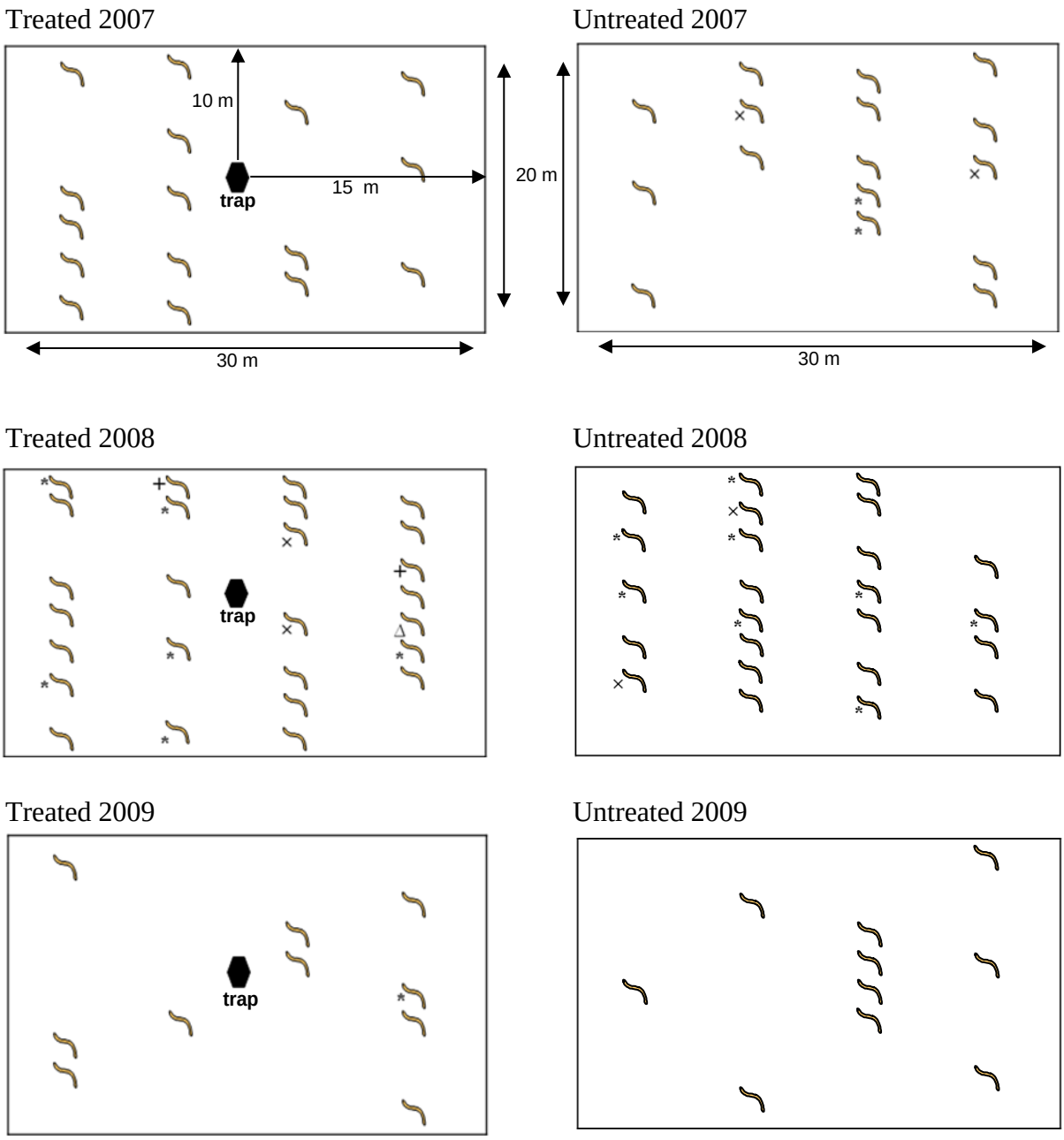

Figure 34: Wireworm (*Agriotes lineatus*, *A. obscurus* and *A. sputator*) catch sites by soil sampling in treated and untreated plots (2007-2009). Treated = sex pheromone traps to catch adult click beetles to control wireworms; Untreated = no sex pheromone traps for wireworm control.

∗ = 2 wireworms at same site, × = 3 wireworms, + = 4 wireworms and ∆ = 5 wireworms.

4.4.3 Wireworm damage

Wireworms tunnel into plant seeds and potato tubers leaving small, round holes on the surface and narrow tunnels running into the tuber flesh (Figure 35). The infestation with 1-2 holes per tuber is enough to reduce yield quality. Wireworm tunneling also creates an entry point for plant pathogens e.g. *Rhizoctonia solani* (KEISER 2007) potentially leading to the drycore syndrome.

Figure 35: Types of wireworm damage

A final assessment of wireworm holes in potatoes grown in 2009 after four years removal of male click beetles via pheromone traps did not show any difference to the control. The extent of potato crop damage (1-2 holes per tuber) varied from 36% to 32% in treated and untreated plots, respectively (Figure 36). The percentage of tubers with no holes tended to be higher (48%) in the control compared to the treated (28%) plots. Similarly the percentage of tuber damage with 3-5 and more holes per tuber was not affected by the treatments.

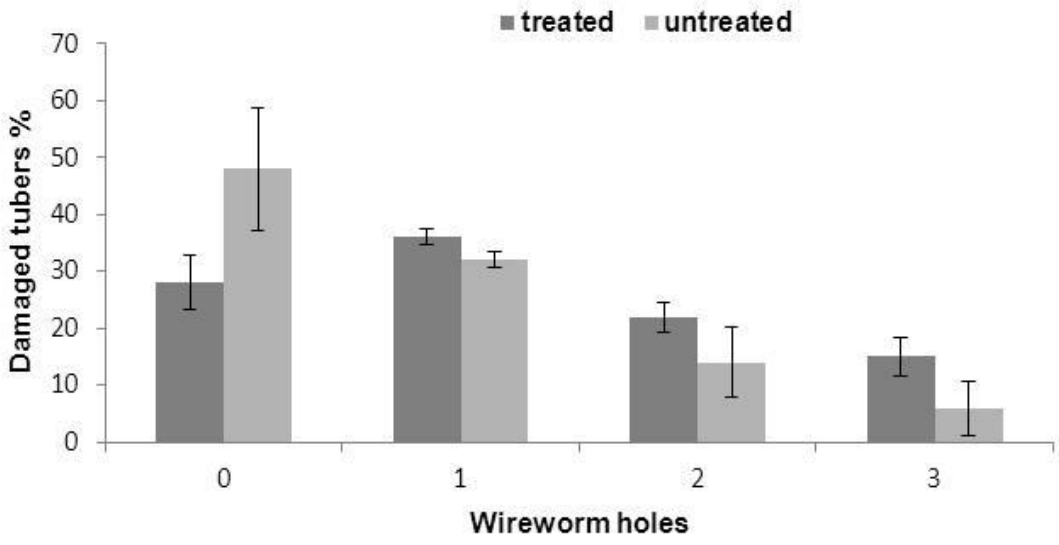

Figure 36: Percentage of tubers with wireworm damage in dependence of the crop in pheromone treated and untreated plots, Tukey's test (α = 0.05). Damage scale: 0= 0, 1= 1-2, 2= 3-5 and 3= >5 holes per tuber.

4.5 Effect of Naturalis (*Beauveria bassiana*) as a biological control for wireworms

In this part of the thesis the effect of the entomopathogenic fungus *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) strain ATCC 74040 against wireworms was evaluated in laboratory and field experiments. The results presented here focus on the wireworm's mortality and percentage of potato tuber damage assessed in the *B. bassiana*-treated area.

4.5.1 Laboratory experiment

The wireworms (*A. lineatus* and *A. obscurus*) mortality to *B. bassiana* was evaluated in a four-week laboratory bioassay. In general, mortality was observed in all three treatments at different extents (Figure 37). The mortality rate in high number of wireworms treatment (5 wireworms per box) was significantly higher than low number of wireworms (3 wireworms per box) and control (2 wireworms per box) treatments. The mortality in high number of wireworm boxes noted 50% compared to low number of wireworms and untreated boxes where death rate was 17% and 13% respectively. Similar percentages of mortality were achieved in both *Agriotes* species and different ranged instars. Seed germination also showed no effect in all three categories.

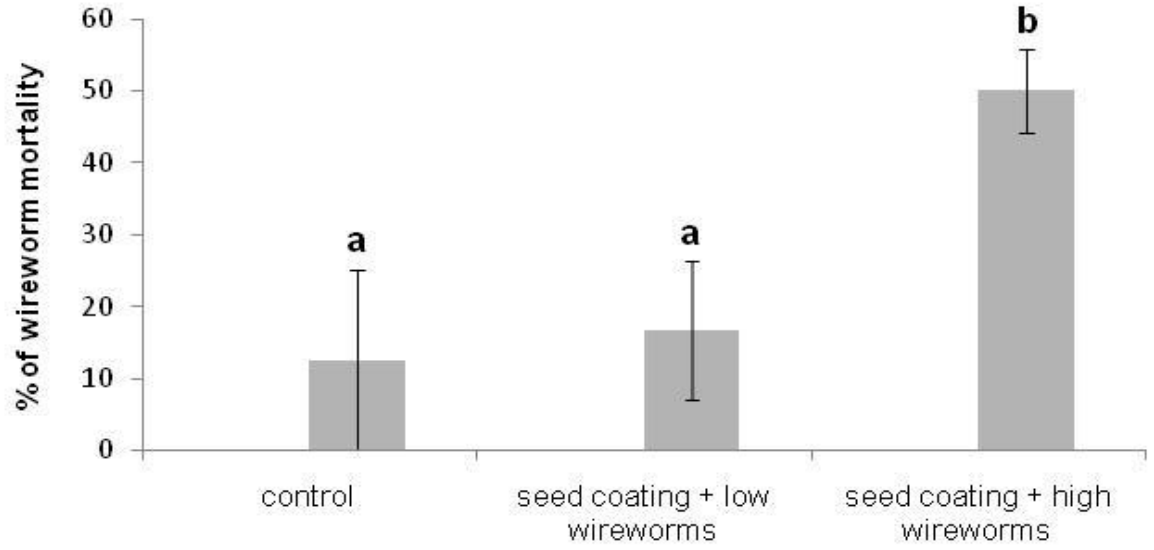

Figure 37: Mean percentage mortality (±SEM) of *A. lineatus* and *A. obscurus* treated with naturalis (*B. bassiana*). Data show the mean of four replicates for each treatment. Means with the same lowercase letters are not significantly different at $\alpha = 0.05$ (Tukey-test).

4.5.2 Field experiment

The wireworm infestation in potato tubers was quite low and showed no significant difference in all treatments. The damage in the plots of naturalis (*B. bassiana*) whole surface application was 1.25%, followed by furrow application (1.50%) and untreated plots (1%) (Table 17).

Table 17: Percentage of tubers damaged by wireworms in different test cases treated with naturalis (*B. bassiana*) in the field known to have elaterids infestation.

Test cases	Treatment (Active ingredient)	Dose	No. of applications	% of damaged tubers
1	Naturalis *B. bassiana* (whole surface)	2.5 L/ha	2	1.25
2	Naturalis *B. bassiana* (rows)	2.5 L/ha	2	1.50
3	Untreated	-	-	1.00

5 Discussion

In order to develop non-chemical control strategies against wireworms, comprehensive knowledge on the biology of this pest is needed. In our work we studied the larval development and the use of pheromone traps for male trapping. We focused on *A. lineatus* and *A. obscurus* since both are known to be the prevailing species in Germany and since detailed knowledge on the biology of both species is still lacking in literature.

5.1 Biology of *A. lineatus* and *A. obscurus*

The life cycle was studied under laboratory and rearing cage conditions. Results showed significant differences in larval development under both rearing conditions. The larval stage had a significantly longer developmental duration compared with the egg, puparium and adult stages for both *Agriotes* species. In our study the first eggs of *A. obscurus* were found in April both in laboratory and in the field. However, earlier oviposition cannot be completely excluded since adults used in this study were first collected in April but may have been active prior to this. In contrast to our findings the oviposition of *A. obscurus* started one to two months later (May and June) in investigations carried out in the UK (FORD 1917, ROBERTS 1919, 1921, EVANS & GOUGH 1942, BRIAN 1947). However, no clear experimental background including weather conditions have been mentioned in their study. Similarly the oviposition of *A. sordidus* started in May in a study carried out in Veneto, Italy (FURLAN 2004). The later oviposition trend of *Agriotes* species both in Italy and UK is against the theory that warm **temperature** causes earlier oviposition, since the average temperature in Veneto (Italy) is (13.2°C) higher than in Bonn Germany (10.3°C). However, species specific habitat requirements or environmental factors and late emergence of adults in spring are likely to affect oviposition trend as well. The early oviposition of *A. obscurus* in our study is probably due to better adaptation of species to cooler climate in Germany.

Eggs were laid either singly or in small clusters, and usually within the protection of grass or deep in moist soil which minimizes the risk of desiccation. In our study up to 39 eggs were laid in a single cluster. Similar findings on oviposition patterns (singly and small clusters) of *A. obscurus* were observed in different studies carried out in UK (ROBERTS 1919, MILES 1942). Similarly *A. sordidus* laid eggs in batches of 3 to more than 30 individuals in a study carried out in Italy (FURLAN 2004). The friability of the soil sometimes makes it difficult to say whether the eggs were always laid coherently or in close proximity. It was not possible to assess total number of eggs laid per female since the females used were captured from outside the field rather than newly emerged from rearing vials. For *A. obscurus* MILES (1942) recorded 40-100 or more eggs per female compared to BRIAN (1947) who counted 30-150

eggs with a significant effect of crop on progeny. For *A. ustulatus* the total number of eggs laid per female amounted 52-140 (FURLAN 1996).

In our experiments under standard conditions at 20°C in the climate chamber *A. obscurus* embryonic development was completed on average after 22.5 days. Similarly in the USA, *A. lineatus* and *A. obscurus* eggs hatched after 3-4 weeks (ANDREWS et al. 2008). In contrast embryonic development of *Agriotes* species tended to be longer and eggs hatched after 4-6 weeks in the UK depending on temperature (FORD 1917, EVANS & GOUGH 1942, MILES 1942). FURLAN (1996) found that embryonic development of *A. ustulatus* was inversely related to temperature, taking 45 days at 15°C, 23.9 days at 20°C and 13 days at 29°C. In the current study the majority of the wireworms hatched in May and the percentage hatchability ranged between 90 and 100%. Similar percentage (95-100%) of egg hatchability was recorded for *A. ustulatus* in Italy (FURLAN 1996).

Young larvae need root material to survive and grow as they have a low level of resistance to starvation (FURLAN 2004). According to our observation *A. obscurus* larvae died without food within 4-5 weeks after hatching. Similarly in another study the majority of the young larvae (*A. ustulatus*) died of starvation within 30 days in the absence of food (FURLAN 1998). In our experiments the mortality of young *A. obscurus* larvae (1^{st} and 2^{nd} instars) was 35% three weeks after hatching and reached 56% before first overwintering when fed with grass roots (*Lolium multiflorum*). In another study the mortality of young *A. sordidus* larvae after 40 days of hatching was 70% when fed with corn seedlings and 33% when fed with ryegrass respectively (FURLAN 2004). Despite sufficient food material the mortality of young sensitive larvae (1^{st} and 2^{nd} instars) might attributed to low resistance against environmental factors or got stress while man handling (collecting for measurements at each assessment). The other possible reason of young larval mortality was the process of exuviation of 1^{st} and 2^{nd} instars to the next instars, which lasted for 10-15 minutes. Similar larval death rate (about 80% of 2^{nd} instar) during exuviation process was noted by WANG et al. (2011) in a biological study of dessert beetle *Microdera punctipennis*.

In total 8-11 instars were identified in *A. obscurus* under the standard rearing conditions (20°C) by the number of moults and frequency distribution of larval body parameters. At the same time 13 instars for *A. obscurus* were recorded to date in the field conditions as the development in the field was not completed so far. In contrast, ROBERTS (1921) and KLAUSNITZER (1994) indicated only eight instar stages for *A. obscurus* larval development. Similar number of larval instars were observed for *A. sordidus* (8-13) and *A. ustulatus* (11-13) development at a constant temperature of 25 and 29°C in Italy (FURLAN 1998, 2004). KOSMACEVSKIJ (1955) stated 14-15 larval instars for *A. ustulatus*. Variability in larval instars among *Agriotes* species might be due to different soil temperatures, duration of overwintering

period and the feeding material provided. According to FURLAN (2004) more instars tended to be found when larvae had not been allowed to stay at low temperatures during winter period.
There was high variation noted in *A. obscurus* larval size at different stages in the current and previous studies. The absolute values, e.g. for head width of L1 were lower in our study (0.20 mm) compared with earlier data (0.75 mm) from FORD (1917), 0.40 from ROBERTS (1919) and 0.28 mm from KLAUSNITZER (1994). Similarly the average length values of first instar stage in the current study (3.66 mm) are comparable with those reported by FORD (1917) (7.00 mm) and ROBERTS (1921) (2.50 mm). According to FURLAN (1998, 2004) the size of 1st instar larvae of *A. ustulatus* and *A. sordidus* was 0.19 mm and 0.18 mm respectively. There was gradual increase in the head size of following instars reaching to 1.05 mm at the 8th instar stage in our study. However, in another study the head width of *A. obscurus* 8th instar stage (final instar) recorded was 1.57 mm (KLAUSNITZER 1994). On the other hand the larvae of *A. ustulatus* and *A. sordidus* reached to 0.75 mm and 1.14 mm size respectively at the 8th instar stage (FURLAN 1998, 2004). The final instar *A. obscurus* larvae in our study (11th stage) reached a size of 1.46 mm compared to final instars of *A. ustulatus* and *A. sordidus* (13th stage) with 1.39 mm and 1.75 mm respectively. These variations in the size of larval instars are likely due to species specific or phylogenetic differences (within the same species).
The larvae usually reach maturity in late summer at which point they burrow deep into the soil and pupation occurs in pupal cells. After three to four weeks depending on soil temperature they become adults. In our study at 20°C the *A. obscurus* pupal stage lasted for 15.4 days. Similar findings on *A. obscurus* pupae were noted by FORD (1917) and ROBERTS (1919) in UK with no abiotic informations. Our data is also in agreement with that of FURLAN (2004), who observed 16 days at 25°C for *A. sordidus*. In contrast *A. ustulatus* pupae developed into light colored adults in 13 days at 20°C and 6.5 days at 29°C (FURLAN 1998).
The sex ratio of newly emerged adults of *A. obscurus* in the present study was 72% males and 28% females. This proportion is just indicative since only 14% of the total population developed into adults in the current study. In contrast the sex ratio of newly emerged adults of *A. ustulatus* and *A. sordidus* was 1:1 in Italy (FURLAN 1996, 2004).
Wireworms often spend many years in the soil and life cycle duration depends on weather conditions that influence the growth and development of insects. The effects of temperature on insect biology are often substantial especially in temperate regions where most of the insects have their growth period during the warmer part of the year (BALE et al. 2002). Variations in the heat sum required to complete wireworm life cycle were noted in the previous and the current studies. In our study of *A. obscurus* under laboratory conditions the average heat sum required (above a base of 9°C) for egg development was about 248 DD, for larvae 9,248 DD, for pupae 170 DD and for the complete cycle from egg to adult about 9,666 degree days. On contrary the heat sum required to complete the life cycle of *A. ustulatus* was

4,156 DD and for *A. sordidus* 3,900 DD respectively (FURLAN 1998, 2004). The degree days reported by KOSMACEVSKIJ (1955) for the whole life cycle of *A. ustulatus* was 5,500 (above a base of 10°C). The average heat sum requires completing life cycle for *A. obscurus* in our study is higher than the heat sum values for *A. ustulatus* and *A. sordidus*. The differences between these results and the present are species specific and due to different temperature used during the study (20°C vs 25 and 29°C). Furthermore, the differences are due to the calculation of heat sum above a base of 10°C, and extended duration of larvae overwintering resulted in lower heat sum values in previous studies. *Agriotes* species requires less time to complete their development at high temperature. This is in accordance with our results where *A. obscurus* development in laboratory at high temperature (20°C) was instantaneous (6.2 instars after first year) than field conditions (4.7 instars) where annual soil average temperature was 11°C.

We have noted that even if sufficient soil moisture and food were present, the average heat sum required completing each larval instar gradually increased with the age of the larvae. The final instar took about eight times more time (1,587 DD) compared with the first instar (190 DD). We noted a steady increase in heat sum for the development of *A. obscurus* larvae under laboratory conditions up to the first overwintering (6^{th} and 7^{th} instar stage) with a slight decrease after overwintering in 8^{th} and 9^{th} instar stages and again a rise for final instars. In contrast, FURLAN (1998, 2004) found a gradual increase in heat sum throughout the larval development of *A. ustulatus* and *A. sordidus* species. Under laboratory conditions, part of the population transformed into adults already in the second year after egg-laying, completed their life cycle (from egg to adult) in 14 months with only one overwintering and two calendar years. Similarly other authors have suggested different duration for the *A. ustulatus* life cycle in different regions; TOTH (1984) suggested 5 years for Ukraine and 4 years for Southern Europe, JAGEMANN (1955) 2-3 years, MASLER (1982) 1-3 years, KOSMACEVSKIJ (1955) 4 years and HINKIN (1983) 2 years. The life cycle duration can also increase to more years if no food is available for long periods during the development (FURLAN 1998).

In our study life cycle development noted for *A. lineatus* was similar to *A. obscurus* in rearing cages with only slight differences in instar stages, population density and overlapping stages at some phases of the cycle. Likewise the majority of the literature states that *A. obscurus* and *A. lineatus* have a similar biological development at comparable site conditions (KLAUSNITZER 1994), similar habitat preferences (CAMPBELL 1937, LEES 1943a) and similar response to environmental stimuli (KUDRYAVTSEV et al. 1993). This similarity in biology and ecology reiterates both species as an *Agriotes* pest complex.

Apart from temperature another important factor which affects larval development is the **soil humidity**. In our experiment constant humidity was maintained in the laboratory, while soil moisture was not investigated in the field. The role of humidity needs to be investigated in

relation to temperature as wireworms have specific moisture and temperature preferences (CAMPBELL 1937). Both temperature and moisture have been shown to affect wireworm development in terms of biochemical reactions, physiological process and movement in the soil. A continuous increase of **soil temperature** encourages wireworm activity in dry substrates. When food is available there is less movement, possibly because food itself may be a source of moisture (CAMPBELL 1937, LEES 1943a). This is well supported by increase in average soil temperature (5 cm deep) during the last 49 years (1961-2009) in our study region from 10.2°C to 12.1°C (DEUTSCHER WETTERDIENST 2010). According to our findings and previous biological studies of *Agriotes* species the wireworms development rate strongly depends on soil temperature (FURLAN 1998, 2004) and any increase has resulted greater incidence of wireworm problem especially in potatoes to seek moisture from tubers (JANSSON & LECRONE 1991). The impact of climate change on the risk of wireworm damage and even epidemics should be considered in a broader ecological perspective in future assessments. Better definition of the conditions under which wireworms are more active will help in understanding how the insect interacts within its environment, ultimately leading to integrated pest management tactics that can exploit these interactions.

5.2 Range of attraction of sex pheromone traps

For the insect species that have long soil dwelling larval stage, non-farmed habitats may provide the transitory areas to complete development and avoid mortality effects caused by control practices (GOUGH & EVANS 1942, BLACKSHAW 1988). This spatial distribution may be of particular importance to insects whose life cycle exceeds more than one year including species such as *Agriotes* wireworms, the larvae of click beetles. Wireworms are particularly difficult to control due to their habitation of the soil, long life cycle, problematic soil sampling, overlapping generations and incomplete knowledge of their ecology. The development of sex pheromone traps for a range of species in the genus *Agriotes* (TOTH et al. 2003) presents a monitoring strategy via mass trapping of adult males. The use of sex pheromone traps has been successful in integrated pest management programmes and it targets adults which are the most accessible and mobile stage and determines species range and rate of spread. Pheromone traps efficacy concerning effective attraction radius (BYERS et al. 1989), sampling range, range of stimulation and range of attraction (WALL & PERRY 1987) for male pea moth (PERRY & WALL 1985, PERRY et al. 1988), male pine sawfly (ÖSTRAND et al. 2000) and *Agriotes* beetles (HICKS & BLACKSHAW 2008) were successfully assessed and quantified in recent studies.

In the present study, we have shown that the range of attraction of pheromone traps and the percentage recapture of *A. lineatus* and *A. obscurus* are comparatively low, significantly decreasing with increasing release distances. On average, the recapture did not exceed 40%

for either beetle species, indicating that, on average, 60% of the beetles were lost, killed or attracted to-and chose to mate with female beetles. A significant decrease of the recapture with increasing release distance for three *Agriotes* species was also noted by HICKS and BLACKSHAW (2008). In their studies, the recapture of *A. lineatus* and *A. obscurus* were, however, higher at short release distances compared with the findings of the present study. The comparatively low overall recapture in the present studies may have been negatively affected by stress (painting and handling) and climatic factors, particularly by repeated rainfall, which was high (>100 mm) in four out of five trials. Studies have shown that **temperature** and **humidity** play an important role in thermoregulation of insects (KROGH & ZEUTHEN 1941, HAUFE 1966). According to HAUFE (1966) all insects will in a gradient, choose a humid environment if desiccated and a dry environment if saturated. Thus, humidity or high temperature may either prohibit or induce flight of *A. lineatus* and *A. obscurus* beetles and affected the outcome. The other possible reason is the sensitivity level of pheromone emissions in the field [i.e. pheromone sensitivity tends to decrease over time, whereas natural female pheromone release remains at the same level; personal observation in the present long-term experiment].

Averaged over all distances two-thirds of the recaptured beetles were recaptured within the first 3 days after release, emphasizing the immediate response of the males to the pheromone plumes. Similarly, ZHANG & SCHLYTER (1996) concluded that 90% of their recaptures of the fall webworm moth *Hyphantria cunea* occurred during the first night after release. The number of beetles caught was significantly greater for beetles released at short (2 and 5 m) compared with longer distances (20 or 60 m), suggesting that the perception of the pheromone plumes is mainly restricted to a short range (between 15 and 20 m). Approximately 10% of the beetles released at a distance of 60 m from the traps were recaptured. The recaptures at this distance were low during the initial days after release and tended to increase after 12 days. We assume that pheromone plumes did not reach males at a distance of 60 m. It is likely that beetles released at 60 m moved randomly towards the traps, thereby detecting the pheromone plumes. This assumption is supported by recent findings in a study by SCHALLHART et al. (2009) on the natural dispersal of *A. obscurus*. According to their experiments, males of *A. obscurus* were able to migrate up to 80 m. In accordance with HICKS & BLACKSHAW (2008), the positioning of the traps along the prevailing wind direction had no effect on the recapture of click beetles. A preliminary conclusion from the present study postulates that *A. lineatus* and *A. obscurus* were responding in a similar way to the pheromone traps under the same environmental conditions. The fact that *A. lineatus* and *A. obscurus* are quite similar in their response to environmental stimuli is not surprising. As both these species coexist in similar habitat and have very close chemical pheromone components compared to other similar species (KUDRYAVTSEV et al. 1993). These findings are in contrast to those of

HICKS & BLACKSHAW (2008), who noted a significant effect of the species, with *A. lineatus* showing higher recapture than *A. obscurus*. These authors, however, used a slightly different method with increased releases of individuals at wider distances, at the same time as working with a smaller amount of total beetles released (60 versus 300 individuals per species and trial). In the present study, the percentage recapture of *A. lineatus* was higher than *A. obscurus* in four out of five trials. When excluding trial 4 (Table 10, see page 62) from the calculations, the overall recapture was 42% for *A. lineatus* and 35% for *A. obscurus*. These values are related with those reported by HICKS & BLACKSHAW (2008) with total recaptures of 39% (*A. lineatus*) and 27% (*A. obscurus*). There is no obvious explanation for the high recaptures of *A. obscurus* in trial 4. Trial 4 was, however, conducted when the grass was tall. It is possible that *A. obscurus* beetles crawled up to the tops of the leaf blades before taking flight to the pheromone trap. *Agriotes obscurus* is known to take flight from thick grass blades (CROZIER et al. 2003).

The probability of recapture from a specific release distance represents important information for trap spacing. A more accurate approach may be given by calculating the maximum sampling range and the effective sampling area of the traps. The maximum sampling range indicates the distance from which an insect can reach the trap by a given time. The effective sampling area indicates the theoretical area around the pheromone trap from which all beetles will be trapped. The maximum sampling range and the effective sampling area were calculated using the same approach employed by TURCHIN & ODENDAAL (1996) and HICKS & BLACKSHAW (2008) for *A. lineatus* and *A. obscurus* using the log *r* to recapture relationship (Table 12, page 67). Compared with the values reported by HICKS & BLACKSHAW (2008), the calculated effective sampling areas in the present study were smaller. The high values for the effective sampling area calculated by HICKS & BLACKSHAW (2008) may also be attributed to the use of the log *r* to log *P(r)* relationship, which was poorer fit to the data of the present study. When calculating their data with the log *r* to *P(r)* relationship, the maximum sampling range and the effective sampling area (38.85 m and 933 m^2, respectively, for *A. lineatus* after 12 days) are corresponding with the values reported in the present study. The use of sex pheromone traps for mass trapping has been successfully applied for the eradication of a range of agricultural pests before reproduction or crop damage (EL-SAYED et al. 2006). With respect to any approach targeted on preventing mating via mass trapping of males, accurate knowledge of the range of attraction of the pheromone traps is indispensible. The results obtained in the present study provide an approximate estimation of the trap density required to remove male beetles from the field, assuming that all captured beetles will not have mated. On the basis of the estimated probabilities of recapture, a maximum distance of 20 m between individual traps would be needed to permit substantial mass trapping. In that case, some 25 traps/ha would be needed to theoretically reduce the

male click beetle population by more than half. When estimating the number of traps needed via the maximum sampling area, ten traps (for *A. lineatus*) and seven traps (for *A. obscurus*) would be needed.

5.3 Use of pheromones for male trapping

The present long-term study was to assess, whether regular pheromone induced male mass trapping of three key *Agriotes* species over five years results in a decrease of soil wireworm abundance and subsequently to reduced damage in potatoes. The method is based on the principle that the Elateridae only seem to disperse locally (although this has not yet been fully investigated) and the number of adults trapped can give an indication of the local population of wireworms in the soil (PARKER & HOWARD 2001). The use of sex pheromone traps is still considered an appealing method and can be deployed easily in the field, thus reducing the application of pesticides. Any control achieved with adult click beetles before egg laying has the potential to decrease the number of eggs that give rise to the next generation of wireworms and can also be used to forecast future outbreaks and devise control measures (TOTH et al. 2002).

Monitoring *Agriotes* species (*A. lineatus*, *A. obscurus* and *A. sputator*) with species-specific pheromones gave a good indication of presence and peaks of these populations (ESTER et al. 2001). In the present study we monitored *Agriotes* species for five consecutive years (2004-2008) and trapped over twelve thousand beetles with different flight peaks and dynamics. There was gradual increase in the number of trapped beetles (*A. lineatus*, *A. obscurus* and *A. sputator*) from 2005 onward until the last assessment year (2008). Similar population increase of *A. ustulatus* and *A. rufipalpis* species trapping was recorded by TOTH et al. (2001) in their long term experiments in Hungary. They caught 533 beetles in the first year of trapping to a dramatic increase of 5,349 beetles in third year compared to 2,372 to 2,986 beetles in our experiments respectively. KARABATSAS et al. (2001) also observed gradual increase of *A. rufipalpis* population from 285 to 6,722 beetles during their four years study in Greece. The steady increase of male trapping in the present study may have been due to the immigration of gravid females in to pheromone-treated areas from the surroundings. The migration of insects might occur to seek food, locate mates, find oviposition sites, wind dispersal and escape predation or harsh environmental conditions (CHAPMAN 1975). The main peaks and notable capturing was observed in the first 4-8 weeks after trap installation in successive years for all three species. In the present study the maximum flight peak was noted with 154 beetles per day for *A. lineatus* in 2007 and 68 for *A. obscurus* in 2006. These values are comparable with those reported by GOMBOC et al. (2001) with 150 adults of *A. lineatus* and 45 of *A. obscurus*. In contrast KARABATSAS et al. (2001) captured a high number of *A. rufipalpis* in early April with more than 1,000 beetles in his long term experiment.

The development of insects is strongly affected by weather conditions (ZASLAVSKI 1988) and has the potential to affect the population dynamics and intensity in agricultural system (STRAND 2000, BOMMARCO 2001). The available data include only a period of 5 years, which may be too short a period to get definite results. However, during this short period study there were some fluctuations noted in trap catches as well as **temperatures**. There were no indications that weather conditions (temperature and rain) have a decisive effect on the size of trap catches, though some minor impacts of temperature on flight peaks of *Agriotes* species were observed. In 2006 the first flight peak of *A. lineatus* and *A. obscurus* was observed at a sum of 691°C temperature while 1,861°C for *A. sputator* (Table 15, page 73). Similar flight patterns were noted at corresponding sum of temperatures in 2007 for all species, while no significant effect of temperature was noted in 2008.

Considering the mass trapping method, their effectiveness is most importantly depending on the density of population present in the area (TREMATERRA & GENTILE 2010). Presumably, in our experiment a highly efficient trapping system has been established, but accurately assessing the effects of mass trapping treatment as a component of pest control still remains the problem. Several authors (BAKKE 1989, VITE 1989) have postulated positive effects of mass trapping as part of integrated pest management system. TREMATERRA & GENTILE (2010) reported mass trapping of 54,170 male Mediterranean flour moth *E. kuehniella* (Lepidoptera: Pyralidae) in five years led to reduction in chemical treatments. However, in their experiment the catches of male Mediterranean flour moth was done inside the mill, i.e. in closed environment contrasting our trapping done in open field. Similarly, captures of *I. duplicates* (Coleoptera: Scolytidae) between 0.5 and 1.7 million per year strongly reduced average coniferous tree mortality to 17% in a 20 years record (SCHLYTER et al. 2003). Furthermore, CORK et al. (2001, 2003, 2005a) have shown that mass trapping of *L. orbonalis* (Lepidoptera: Pyralidae) resulted in a 50% increase of brinjal fruit yield without using insecticides. Mass trapping is one of the long-standing approaches to direct control of insects for population suppression and eradication (STEINER 1952), but is partly successful to *Agriotes* species based on mark-release-recapture assessments (HICKS & BLACKSHAW 2008, SUFYAN et al. 2011). However other authors had no success with this method in the field (WESLIEN 1992, PFEFFER & SKUHRAVI 1995).

Pheromone mediated mating disruption is also an alternative strategy in biological control programs, which has been successful in many agricultural pests. The reproductive potential (both in fecundity and fertility) of lepidopterous pests (*A. velutinana*, *P. pyrusana* and *C. pomonella*) was adversely affected by reducing the available number of males as a consequence of mating disruption (VICKERS 1997, STELINSKI & GUT 2009). Pink bollworm, *P. gossypiella* (Lepidoptera: Gelechiidae) is a difficult pest to control in cotton growing areas in the world causing losses in both yield and quality. Mating disruption has proven to be

successful and yields were enhanced while crop damage was only 5% in treated compared with more than 30% in conventionally managed fields (DOANE et al. 1983). Similarly by mating disruption a significant continuous decrease in egg population of *P. unionalis* (Lepidoptera: Pyralidae) was observed during respective years in Egypt (HEGAZI et al. 2007).
According to our data a large number of adults of each *Agriotes* species were trapped in sex pheromone traps, whereas the proportion of wireworms of the same species (from the same fields) was not necessarily equivalent. The misleading picture of adult male species trapped by sex pheromone traps and below ground wireworm distribution (for instance 1,213 *A. sputator* adults vs 15 larvae detected in our study) has important implications in cases where the monitoring of a pest is carried out on a different life stage to that causing the damage. Possibilities are that eggs are deposited elsewhere in fields i.e. in field margins which were not sampled in this study, or adults are better able to disperse and/or attracted to sex pheromones from a wider area than other species, meaning they are moving in from other non-sampled areas. The non-*Agriotes* species trapped were not always found in large numbers and were very low relative to *Agriotes* wireworms, but this may suggest there are certain site characteristics or other factors, which affect the presence, abundance and distribution of these species.
In our study, it is obvious that the use of pheromone traps for controlling adults as a surrogate for larvae still needs justification. The wireworms trap counts and their spatial distribution in treated and untreated plots showed no significant effect and the present study failed to establish any adult-wireworm relationship. Similar findings of poor relationship between catches of male (*A. lineatus* and *A. obscurus*) beetles and wireworm counts were noted by BLACKSHAW & VERNON in 2006. However by accurate pheromone trap spacing there were some signs of adult-wireworm relationship of *A. obscurus* recorded by BLACKSHAW & VERNON (2008) in a spatial relationship of *Agriotes* species. One possible reason of proportional wireworm findings on our experimental site with only two replications and a comparative smaller space of 50 m between the treated and untreated plots. Although we selected two control plots without pheromone traps at a distance of 65 m to the pheromone traps but seems to be small due to larger spatial distribution of *Agriotes* species. Similar findings of natural dispersal were noted by SCHALLHART et al. (2009), when males of *A. obscurus* migrated up to 80 m and more. Comparable results were obtained in our mark-release-recapture experiment where beetles were recaptured in pheromone traps placed at 60 m from their point of release (SUFYAN et al. 2011).
According to FURLAN (1996) *A. ustulatus* populations in Italy oviposit 5 to 7 days after adult emergence, and the oviposition period lasts 2 to 4 days. This would suggest that the vast majority of oviposition would occur two weeks after adult emergence, and oviposition activity would be expected to closely follow adult emergence trends by approximately two

weeks. After oviposition, flight activity by adults is of much less importance in terms of population management, since adults cause little damage to crops. If we assume the sex ratio 1:1 reported by FURLAN (1996), it would then be plausible to expect that trap catch data would closely approximate adult emergence and oviposition trends with a lag time of two weeks. Since male beetles typically mate more than once in their life, a large number of male populations must be removed at once to produce an effect. As wireworms dwell three to four years in soil, and once a field is inhabited population continue to exist for many years and cohort different generations of trapped beetles (BLACKSHAW & VERNON 2008).

The missing effect of male mass trapping on wireworm abundance also resulted in no effect on tuber damage. Tuber damages by wireworms however are a key problem in organic potato production. In an investigation under the Federal Organic Farming program, an increase of wireworm damage in Germany was observed over the last few years (VUB-RING ÖKOLOGISCHER LANDBAU E.V. – ÖKORING 2009). The amount of damaged potatoes was about 7%, and their damage to tubers reduces crop quality rather than yield. Moreover, in North Rhine-Westphalia (Germany), a minimum of 11% to a maximum of 80% of potato tuber harvest has been down-graded or rejected outright because of wireworm injury, resulting in substantial economic losses (SCHEPL & PAFFRATH 2007). The potato damage assessment carried out after the five years removal of adult click beetles in our study showed no significant effect in both treated and untreated plots. The overall damage in pheromone treated area was amazingly high compared to untreated area. PARKER (1996) found wireworm damage even in fields where very few wireworms were detected; on the other hand low wireworm damage was recorded on fields with high infestation level. There are some factors which affect the wireworm damage level including adult immigration, egg laying and their survival, wireworm population age structure (larger wireworms may be more damaging), soil temperature and moisture, date of harvest and may be potato cultivar. The potential of pheromone traps currently used for mass trapping of male click beetles with subsequent wireworm reduction still remains hypothetical. However, pheromone traps are sensitive enough to detect low-density populations, and are, therefore, effective for tracking invasive species in the establishment phase. The fact that adults may be ubiquitous in the field and can be attracted from non-farm habitats, whereas lacking movement of *Agriotes* larvae in the soil requires a further research for a clear relationship between trap catches and subsequent wireworm abundance in the soil.

5.4 Effectiveness of *Beauveria bassiana*

The results of the study have shown that *Beauveria bassiana* has a potential to be used as a biological control agent against *Agriotes* wireworm populations. The wireworms showed sensitivity to *B. bassiana* and significant mortality of larvae were observed in some treatments

of laboratory bioassays. There was significant mortality (50%) of population noted in high number of wireworm boxes comparison to control and low number of wireworm boxes. This suggested that higher exposure to entomopathogenic fungus resulted higher mortality of wireworms. Similarly the potential of *B. bassiana* to control cherry fruit fly and the olive fly has been noted in different laboratory and field studies in Italy (BENUZZI et al. 2007, LADURNER et al. 2008). ORTU et al. (2009) reported that, the bioinsecticide based on *B. bassiana* strain ATCC 74040 can reduce significantly *C. capitata* populations and the number of punctures on fruits. Similarly CHIKWENHERE & VESTERGAARD (2001) observed in their bioassays that *B. bassiana* induced significant mortality of *Neochetina bruchi* stages (eggs, larvae and adults) when they were either sprayed or dipped in different conidia concentrations.

There were no significant differences noted in the percentage of damaged tubers in Naturalis treated and untreated plots. The tuber damage by wireworms was very low in both plots. This might be due to very low wireworms population in the area (no wireworm was observed in a soil sampling done before tuber plantation). The other possible reason of low tuber damage was the other agronomic and abiotic factors that prohibited a wireworms outbreak in the field. Wireworms need many years to develop and infested fields consist of larval populations of mixed ages, stages of development and different *Agriotes* species at the same time. Thus for a viable biological control of the pest, the interplay between biotic and abiotic factors including *B. bassiana* virulence, wireworm behavior and wireworm susceptibility needs to be established. Our laboratory studies suggest that more trials should be carried out and the bioinsecticide based on *B. bassiana* strain ATCC 74040 could be a valuable additional tool to efficiently manage *Agriotes* infestations in both integrated and Organic Agriculture.

5.5 Outlook

Cultural control, e.g. rotation design, is still the most elegant approach to cope with pest and disease problems in Organic Agriculture. According to our findings we can expect the peak of the oviposition of *A. lineatus* and *A. obscurus* for the site conditions of the Southern Rhineland in May. The larvae will hatch after three weeks and during the first year between 4 to 6 larval stages will develop before winter rest. In the first year after oviposition the larvae will undergo another 4-6 instars, resulting in a larval size, which increases the risk of potato damage. The total amount of larval stages varies even within one species and may range between 8-14 instars. Accordingly damaging stages of wireworm larvae will still occur in the soil in the second year after oviposition. With respect to rotation management this would mean that in the two preceding years before potato cultivation no crops should be grown that are favourable for oviposition. Click beetles females prefer to deposit their eggs in fields with

dense vegetation such as grass or grass-clover leys. Our assessments with pheromone traps however have shown that the presence of male click beetles is also high in cereals.

From a theoretical point of view a crop rotation suitable for avoiding wireworm damages should therefore not include grass-clover leys and cereals in the two preceding years before potato growing. Hoe crops such as faba bean, maize or field vegetables are less attractive for oviposition, since during the main period of oviposition (May) the soil will be tilled. Since the inclusion of grass-clover leys is a key factor for fertility building, many organic farmers don't want to exclude this element from the rotation. In that case leys should be followed by hoe crops for two years, even if from agronomic point of view (precrop effects) this option might not be ideal.

Another option for potato rotation design would be to exclude leys and to look for an adequate integration of cereals after potatoes. These crop rotations however may cause serious problems with soil carbon supply and would require high amendments of organic matter such as manure. Both proposed changes in crop rotation design for avoiding potato damage by wireworms still require experimental evidence.

In addition to rotational aspects any option for intensifying soil tillage during the main time of oviposition, i.e. in May, should be considered. Likewise earlier lifting of tuber, whenever possible, is a promising option to reduce wireworm damage in potatoes (NEUHOFF et al. 2007).

The use of pheromone traps to capture male click beetles is an efficient technology. However, these traps can only be used as monitoring tool e.g. for fields where soil sampling or baiting techniques have failed to detect larval infestations. Using pheromone traps for controlling wireworms via mass trapping of adult males is apparently not possible. Another clear drawback of pheromone technique for click beetles is that only the male population can be trapped. The development of pheromones directed at females is therefore expected to become an important complementary tool for wireworms control. Likewise the formulation of female attractant dispensers especially to lure egg-laying females would be useful in sustainable pest management programs.

Finally biological control of wireworms by using e.g. entomopathogenic fungi, such as *B. bassiana* is a promising option. These approaches however need further research for practical implementation.

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7 List of Figures

8 List of Tables