

Sanna Boman

Ecological and Genetic Factors Contributing to Invasion Success

The Northern Spread of the
Colorado Potato Beetle
(*Leptinotarsa decemlineata*)



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"I've always found that sticking your fingers in your ears and humming loudly solves a whole host of problems"

Richard Dean Anderson as Jack O'Neill

ABSTRACT

Boman, Sanna

Ecological and genetic factors contributing to invasion success: The northern spread of the Colorado potato beetle (*Leptinotarsa decemlineata*)

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Yhteenveto: Ekologisten ja geneettisten tekijöiden vaikutus koloradonkuoriaisen (*Leptinotarsa decemlineata*) leviämismenestykseen

Diss.

To prevent or manage invasions of alien species, it is vital to study their invasion potential before they colonize new areas since the control of invasive species is easiest in the initial stages of invasion. I assessed the potential of an invading pest species, the Colorado potato beetle (*Leptinotarsa decemlineata*), to expand north of its current range in Europe from both the ecological and evolutionary perspectives. The results show that European Colorado potato beetle populations still exhibit adaptive genetic variation in life-history traits despite the bottleneck they experienced at the beginning of the 20th century when they reached Europe from United States. Development time showed high heritability indicating that the beetles have the potential to shorten their developmental time, which is necessary in order to survive in the northern latitudes due to the short growing season. Furthermore, the beetles that had the shortest development times were also the largest indicating that development time does not constrain size, which may facilitate colonisation to higher latitudes. Beetles were very cold tolerant as exposing larvae to subzero temperature (simulating night frosts) did not incur mortality. I also show that human-induced selection by insecticides can incur significant costs as the offspring of exposed parents were smaller than, and had an increased metabolic rate in comparison to, the progeny of parents that were not exposed. However, this pesticide-induced selection may also have beneficial effects as the offspring of exposed parents had high relative lipid content, which may, in turn, improve their overwintering success. These dual effects of insecticides should be taken into account when planning the management of this species. Taken together, my results suggest that Colorado potato beetles possess the ability to adapt to the climatic conditions existing north of their current range in Europe.

Keywords: Geographical variation; invasion potential; pest insect; species invasion

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LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original papers, which will be referred to in the text by their Roman numerals I-V. For paper I, I participated in the manuscript preparation. For papers II-V, I took part in planning the experimental design, performed the majority of the work and wrote the first drafts of the manuscripts, which were completed in co-operation with the other authors.

- I Grapputo, A., Boman, S., Lindström, L., Lyytinen, A. & Mappes, J. 2005. The voyage of an invasive species across continents: genetic diversity of North American and European Colorado potato beetle populations. *Molecular Ecology* 14: 4207-4219.
- II Boman, S., Grapputo, A., Lindström, L., Lyytinen, A. & Mappes, J. 2008. Quantitative genetic approach for assessing invasiveness: geographic and genetic variation in life-history traits. *Biological Invasions* 10: 1135-1145.
- III Boman, S., Lindström, L., Lyytinen, A. & Mappes, J. 2008. Life on two edges: potential routes for the northern range expansion of *Leptinotarsa decemlineata* in Europe. Manuscript.
- IV Boman, S., Grapputo, A., Lindström, L., Lyytinen, A. & Mappes, J. 2008. Cold tolerance during the growing season: implications for range expansion potential in *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). Submitted manuscript.
- V Boman, S., Lindström, L., Lyytinen, A. Mappes, J. & Piironen, S. 2008. Cross-generational effects of insecticides on metabolic rate, lipid content and overwintering success. Manuscript.

1 INTRODUCTION

1.1 Species invasion

"Alien species means, with respect to a particular ecosystem, any species, including its seeds, eggs, spores, or other biological material capable of propagating that species, that is not native to that ecosystem"

Invasive species means an alien species whose introduction does or is likely to cause economic or environmental harm or harm to human health"

Clinton 1999

Species invasion is a burning issue in applied ecology since the number of invading species has rapidly increased in recent years mainly due to human impact and global warming (Sax et al. 2005, Lockwood et al. 2007). Most species introductions are human induced (e.g. medicinal plants, the pet trade) and only fractions are natural invasions (Whitney & Gabler 2008). Invading species may have negative impact on native biodiversity (e.g. extinction of native species), cause public health risks and severe damage to agricultural productivity (Kolar & Lodge 2002, Sakai et al. 2001) which can have high economic costs (Pimentel et al. 2000, Pimentel et al. 2005). The need to control and prevent invasions has led to an enormous increase in studies concerning invading species in the last few years (Lockwood et al. 2007).

The biological study of invasions was inspired by C. S. Elton's (1958) book "The Ecology of Invasions by Animals and Plants" in which he argues that there is urgent need to protect nature from invasive species. Moreover, he suggested that we have to understand what factors affect species invasions and try to create a general overview about species invasions. Unfortunately, 50 years after the publication of Elton's book, the theoretical underpinning of invasion biology is still in a state of confusion, in which theories and terminology from ecology to evolution and genetics are loosely linked and used (Sax et al. 2005). Even the basic terminology of invasion biology is

miscellaneous (Colautti & MacIsaac 2004). For example, the terms alien, introduced, non-native, non-indigenous, foreign, and exotic species are all used as synonymous of invasive species (Colautti & MacIsaac 2004, Sax et al. 2005, Lockwood et al. 2007). The use of different terms are often related to specific stages of invasion (see next chapter) and/or whether the invasion is human-induced or has occurred naturally (Colautti & MacIsaac 2004). Despite or rather due to these differences the use of these terms in invasion literature suffers from a lack of consensus.

Studies in invasion biology have been conducted, for the most part, from one of two distinct approaches - the ecological or the evolutionary approach (Lee 2002). The ecological approach examines species invasions by understanding their ecological requirements and applying this information to evaluate the suitability of habitats for their invasion (Heger & Trepl 2003, Lockwood et al. 2007). This approach includes the use of distribution models such as bioclimatic modelling, where the temperature conditions of species' current and native range are compared to possible alternatives (Jeffree & Jeffree 1996, Gaston 2003, Rafoss & Saethre 2003). However, these models are frequently criticized since they do not take into account the possibility that species niche (i.e. suitable environmental conditions) may be larger than the known niche properties of their current range (Holt et al. 2005). The ecological approach also searches the shared ecological characteristics (e.g. life history traits) of invaders in order to predict possible likely invasive species (Lockwood et al. 2007). This approach concentrates on interactions between habitats and species, such as the impact of invasive species on native communities, since invasive species may have a large influence on species present in the invaded area (Heger & Trepl 2003, Lockwood et al. 2007).

The evolutionary perspective in invasion biology emerged from the need to understand species' colonization success in relation to genetic factors (Lee 2002, Parker et al. 2003, Huey et al. 2005). Invading species' evolutionary responses play an important role during the invasion process as they may affect both a species' potential to spread and their interactions with other species and habitats (Huey et al. 2005). Invading populations may undergo evolutionary adaptations due to the different selection pressures (e.g. climate, competition) in the new environment, which may improve their fitness and thus their colonization success. Therefore, when studying species invasions one should take into account, alongside the ecology of given species, differences in their potential to change when arriving in new environments (reviewed in Sakai et al. 2001, Kolar & Lodge 2002, Parker et al. 2003, Gilchrist & Lee 2007). Nevertheless, many - if not most - studies on invasive species have been undertaken from an ecological point of view, while the evolutionary aspects of invasions have been substantially under-explored (Lee 2002, Parker et al. 2003). Current research is, however, changing this imbalance in the literature.

Most biological studies of invasions have focused on successful invasions, but it is good to bear in mind that only 1% of all invasion attempts succeed, according to an estimate by Williamson & Brown (1986) (but see Jeschke & Strayer 2005). This means that the field of invasion biology is still in its infancy

and is mainly a descriptive science (Whitney & Gabler 2008). Thus, there is an urgent need for studies which concentrate on species' invasion potential (i.e. the initial stage of invasion) (Dlugosh & Parker 2008, Whitney & Gabler 2008). To better understand the potential of species to expand their range, more emphasis should be put on studying the interactions between the ecological and evolutionary forces (Lee 2002, Gaston 2003). That is, ecologists should realise that species ecology is largely determined by their genes and geneticists should note that knowledge extrapolated solely from species genetics cannot necessarily explain a species' colonization success without understanding of its ecological attributes (Mayr 1965, Facon et al. 2006).

1.1.1 Invasion process

The invasion process has three main phases: introduction to a new area, establishment, and spread to nearby areas (Vermeij 1996, Kolar & Lodge 2001, Sakai et al. 2001, Novak 2007). In the introduction phase, individuals move from the species' native range to a new habitat, often involving long-distance transportation, even across continents. Mortality is usually high at this stage and only fractions of invading individuals usually survive. During the second phase, the species establishes a viable population in the new habitat (Vermeij 1996, Kolar & Lodge 2001). Between the introduction and establishment stages, there is often a time lag. This "lag time" refers to the time required for local adaptation to evolve and cope with possible inbreeding depression (Kolar & Lodge 2001, Sakai et al. 2001). During the lag time, the population size is often small but increases rapidly as the population better adapts to its new environment. It has been suggested that species which are pre-adapted to the new environment do not have any lag time but are able to almost immediately establish viable populations and rapidly increase their population size in the new environment (Sakai et al. 2001, Lee 2002). The term lag time is also used to refer to the time that populations need to reach the population size necessary for rapid growth and expansion, thus having nothing to do with evolutionary responses. During the third stage of invasion, the population starts to spread to nearby areas (Kolar & Lodge 2001, Sakai et al. 2001, Novak 2007) and pest species' population densities typically are so high as to become a problem (Williamson 2006). Many studies in invasion biology focus on the species establishment stage even though both the eradication and management of invading species would be easiest at the initial stage of invasion (Genovesi 2005).

1.2 Factors affecting species invasion

If we want to predict which species are the most likely invaders, we have to understand which factors limit species range boundaries. Darwin (1859) already noted that the species' geographical distribution area can be constrained by

barriers to dispersal. In this era of globalization, geographic barriers for dispersal are mostly removed by humans due to the shipment of goods. However, barriers to successful invasion also include ecological (abiotic and biotic) and evolutionary (genetic) factors which affect species ability to survive and reproduce in a new environment (Gaston 2003, Sax et al. 2005, Lockwood et al. 2007 and examples therein). There is still debate as to whether species' range boundaries and invasions are limited by only a few or by multiple factors (interactions between ecological and genetic factors). The problem is that it is difficult to conduct a study that simultaneously takes into account all possible factors (i.e. multiple interacting factors) affecting a species distribution. Furthermore, the importance of the limiting factors might be species specific, dependent on the environment or even the population (Lodge 1993, Lee & Gelembiuk 2008). This might make it difficult to find general laws that would explain species invasion (but see Arim et al. 2006).

1.2.1 Ecological factors

Several abiotic and biotic factors can prevent species invasion to new areas such as physical barriers (e.g. rivers, mountains) and climatic factors (Gaston 2003, Lockwood et al. 2007). However, these factors may limit species range boundaries only if the species are not able to adapt to overcome them (see next chapter) (Huey et al. 2005).

One of the major ecological factors affecting species distribution, especially in insects, is temperature (Hoffmann & Blows 1994, Gaston 2003). Other factors known to vary with latitude, such as photoperiod and nutrition, may affect insects' morphology, growth and reproduction (Roff 1992, Zaslavski 1988). For example, development time, size and metabolic rate in insects can vary as a function of latitude (e.g. Roff 1980, Chown & Nicolson 2004, Nygren et al. 2008). Many insect species need a certain threshold temperature to complete their development and, generally among insect species, decreasing temperature increases development time (Chown & Nicolson 2004). Consequently, insect species invading higher latitudes must be able to develop and survive at lower temperatures to successfully occupy these cold areas. Particularly at high latitudes and altitudes, species are often exposed to temperature extremes, such as subzero temperatures, even during the growing season (i.e. night frost). These temperature extremes are often a more important limiting factor determining the species' distribution and survival than are average temperatures (Inouye 2000, Tesar & Scriber 2003).

Species invasion to new areas is also dependent on biotic interactions such as competition, predation, parasitism and host distribution (Sax et al. 2005, Lockwood et al. 2007). One important factor affecting the success of an invading species is their natural enemies, which may prevent species range expansion by influencing their survival (i.e. by keeping population density low). The enemy-free hypothesis states that invading species often perform better in a new area because their natural enemies in their native range do not follow them into the new areas (Wolfe 2002). Moreover, competitive ability has been shown to

increase species' invasion success (Sakai et al. 2001). Invasive species that are able to use local resources more effectively than native species are more likely to establish themselves in the new region (Gaston 2003). The ability to compete well is important especially in communities where native species have occupied most ecological niches (Lockwood et al. 2007). A further factor that may limit the invading species' distribution is the distribution of essential resources (e.g. host plant) (Gaston 2003). For example, without an essential host plant the species may not be able to reproduce and/or develop and thus it will not be able to colonize otherwise hospitable host-barren areas (Gaston 2003).

It has been suggested that disturbance facilitates species invasions (e.g. Lee & Gelembiuk 2008, Lockwood et al. 2007). Disturbance is defined, according to White & Pickett (1985), as "any relatively discrete event in time that disrupts ecosystem, community or population structure and changes resources, substrate availability, or the physical environment". Disturbances may be abiotic or biotic such as drought, fire, insecticides and predators (Lockwood et al. 2007). Disturbance by fire, for example, has been shown to promote several invasions, especially in plants such as the invasive grass *Bromus tectorum* whose invasion speed is accelerated by fire (Lockwood et al. 2007). From an ecological point of view, disturbance may limit or enhance species invasions by changing habitats or the interactions between species and habitats making the habitat itself more (or less) suitable for the invasive species (Lockwood et al. 2007). For example, natural disasters (floods, hurricanes) may change the soil conditions and increase light-availability for species (e.g. plants) thus facilitating the colonization success of certain invasive species (Lockwood et al. 2007). Disturbance may also lead to evolutionary changes in invasive populations if it selects for genotypes that are favourable in the new environment (Lee & Gelembiuk 2008). Anthropogenic disturbance, such as agricultural management with insecticides, for example, may lead to the evolution of insecticide resistance making the pest species more persistent and thus facilitating their invasion (Mallet 1989, Sakai et al. 2001).

1.2.2 Genetic factors

Genetic diversity can be divided into two principal types: neutral and adaptive. Neutral genetic variation is expected to have no effect on individual fitness and it can be analysed using molecular genetic methods (e.g. AFLP, mtDNA) (Dlugosch & Parker 2008a). The amount of neutral genetic variation in a population does not necessarily correlate with the amount of adaptive genetic variation (Lindholm et al. 2005, Dlugosch & Parker 2008a). However, neutral genetic variation can give important insights into the amount of gene flow between different populations as well as species' invasion routes and history (Kolbe et al. 2004, Dlugosch & Parker 2008a). Although much information can be gained from measures of neutral genetic diversity, characterization of genetic variation in life-history traits and the processes generating this variation are likely to be more important in determining potential invasion success (Lindholm et al. 2005). Adaptive genetic variation is affected by natural

selection and it can be studied using quantitative genetics methods (Hoffmann & Blows 1994). For example, species adaptive potential can be assessed in quantitative genetic experiments by analysing the presence of additive genetic variance in important life-history traits (Hoffmann & Blows 1994, see also Lee et al. 2007). The absence of additive genetic variance in important life-history traits may directly restrict species' range (Griffith and Watson 2006) and their potential to cope with the changing world (Hoffmann et al. 2003).

Often, the number of invading individuals (propagule size) may be low, suggesting that the species has suffered from founder effects and/or has undergone a bottleneck during invasion. This means that populations might have lost genetic diversity during invasion, especially the rare alleles (Dlugosch & Parker 2008a, but see Kolbe et al. 2004). It has been shown that loss of genetic variation is a common phenomenon (but see review by Novak & Mack 2005). During the invasion process, species can lose up to 18.7% of their heterozygosity (Dlugosch & Parker 2008a). While bottlenecks may lead to a decrease in neutral genetic variation, they do not necessarily cause an immediate reduction in variation (i.e. loss of adaptive genetic variation) in fitness-related traits (Koskinen et al 2002, Dlugosch & Parker 2008a). In fact, there are a few examples where invading species have shown remarkable ability to adapt even though they have been subjected to a significant loss of neutral genetic variation. The guppies *Poecilia reticulata* in Australia, for example, had high levels of additive genetic variability despite low neutral genetic variability (Lindholm et al. 2005). Another example is the invasive shrub *Hypericum canariense* which has shown potential to adapt and evolve (i.e. latitudinal cline in flowering phenology) despite a decrease in genetic diversity caused by a population bottleneck (Dlugosch & Parker 2008b). This suggests that loss of neutral genetic variation does not necessarily constrain species' potential for adaptation and thus their invasion success (Dlugosch & Parker 2008a). However, a lack of adaptive genetic variation in relevant life history traits may hinder species' potential for local adaptation, since natural selection requires genetic variation in traits (Lee 2002, Kawecki & Ebert 2004). High adaptive genetic variation would allow a more rapid response to selection, enhancing potential to adapt to new environments (Carroll & Dingle 1996, Garcia-Ramos & Rodriguez 2002, Lee 2002) and thus improving colonization success (Parker et al. 2003).

Multiple introductions are more common than a single successful invasion with large propagule size (Memmott et al. 2005). It has been suggested that multiple introductions can increase the genetic diversity of invasive populations (Kolbe et al. 2004, Novak 2007, but see Dlugosch & Parker 2008a), but this increase does not necessarily show up in populations for decades (Dlugosch & Parker 2008a). A population's genetic variability may also increase via gene flow. On the other hand, gene flow may work as a factor restricting local adaptation, especially if the gene flow comes from central populations since such individuals might not be so well-adapted to the less suitable conditions existing on the margins (Hoffmann & Blows 1994). However, both multiple introductions and gene flow are not vital for successful invasion since

the possible increase in genetic variation may not be expressed until the invasive species have already successfully colonized the new region (Dlugosch & Parker 2008a).

1.2.3 Phenotypic plasticity

Phenotypic plasticity means that organisms with certain genotypes may express different phenotypes in different environmental conditions (Bradshaw 1965, Dewitt & Scheiner 2004, Garland & Kelly 2006). These include changes in life history traits, behaviour, physiology, and morphology. Kawecki & Ebert (2004) have proposed that phenotypic plasticity is one alternative to local adaptation. Thus, it has been hypothesized that phenotypic plasticity improves species potential to colonize new areas since the invasive species could respond to different environmental conditions, which would increase their ability to survive the establishment stage (review in Richards et al. 2006 and references therein). Phenotypic plasticity has an especially important role if the introduced population has suffered loss of genetic variation due to a bottleneck and/or founder effects (Richards et al. 2006, Ghalambor et al. 2007). In that case, phenotypic plasticity allows populations to tolerate and adapt to new conditions despite low genetic variation, thus possibly preventing their extinction in a new area (Ghalambor et al. 2007). Therefore, successful invasive species may have more phenotypic plasticity in traits related to fitness than do non-invasive species (Richards et al. 2006). Another proposed hypothesis is that, in a new environment, natural selection may favour genotypes with increased phenotypic plasticity, thus leading to rapid evolution of plasticity in invading species (Parker et al. 2003, Richards et al. 2006). This would, in turn, facilitate successful invasion and colonization (Richards et al. 2006).

Phenotypic plasticity occurs not only within the life-span of a single individual but also across generations (i.e. maternal effects) (Agrawal et al. 1999). Maternal effect means that the offspring's phenotype is affected by the maternal phenotype in addition to the mother's genotype (reviewed in Räsänen & Kruuk 2007). Maternal effects may cause phenotypic plasticity in offspring and thereby allow rapid phenotypic change. Selection pressures (e.g. environmental stress) that influence the mothers may affect traits in the next generation (cross-generation effect). The influence of maternal effects on species invasion success has been understudied even though they may have population-level consequences and affect species invasion to new environments as shown by Badyaev (2005). Species inhabiting marginal regions of their distribution areas are often exposed to a stressful environment, which may have effects on offspring fitness. For example, mothers developing in poor conditions (e.g. with limited nutritional resources) may produce small eggs which may have subsequent effects on offspring development time, size and survival (review in Mousseau and Dingle 1991, Mousseau & Fox 1998, Fox et al. 1999, Holbrook & Schal 2004).

1.3 Implications

Why do so few invasions succeed and what traits make invasive species successful? These are the essential questions for invasion biologists and there has been an intensive search to determine the shared ecological characteristics of invasive species in order to predict possible candidates for new invasions (e.g. Williamson & Fitter 1996, McKinney & Lockwood 1999, Review in Kolar & Lodge 2001, Marchetti et al. 2004). A list of characteristics associated with successful invasion would be beneficial for both prevention and management. Knowledge of these characteristics might make it possible to predict likely-invader species and high-risk environments, which would help in early detection, eradication and control (Marchetti et al. 2004). Based on current knowledge, invasive species are mainly r-strategists; they have small size, high fecundity and early reproduction (Williamson & Fitter 1996, Kolar & Lodge 2001). Furthermore they have a wide distribution area, a history of successful invasions, a good dispersal ability and are resistant to environmental stress (Kolar & Lodge 2001, Carroll & Dingle 1996). Unfortunately, currently available descriptions of successful invasive species are not universally applicable and are mainly restricted to a few taxa (i.e. plants, insects) or restricted geographical areas (Carroll & Dingle 1996). Perhaps invasion biology's lack of a consistent theoretical foundation and inappropriate methods for studying the characteristics of successful invasive species has delayed the achievement of this goal. That different traits may be important at different stages of invasion, for example, has been underestimated (Kolar & Lodge 2001), and studies do not usually compare data from successful and failed invasions (but see Marchetti et al. 2004), which might be one key to solving this goal. Moreover, it is only in the last few years that quantitative genetic methods have been taken into account when predicting potential invasive species (Kolar & Lodge 2001) even though genetic factors have an important role during the invasion process (Huye 2005). Studies on potential invasive species should be conducted with more emphasis on the interactions between ecological and genetic factors since both ecological and genetic factors play an essential role (see previous chapters) during the invasion process and determine invasive species' invasion potential and further success in the new area.

1.4 Aim of the study

In this thesis, I combined both ecological and genetic methods to gain a more comprehensive understanding of the invasion potential of a species before it actually invades. Assessing species invasion potential to new areas gives us tools to recognize potential invasive species, such as traits which contribute species invasion success, and helps us recognize favourable habitats for invasion.

An ideal species to study how genetic and ecological factors contribute to species invasion success is the Colorado potato beetle (*Leptinotarsa decemlineata*). This species allows us to assess the potential of an invader to further expand its range in the introduced area, an under-explored field of study in invasion literature. The beetle has a known invasion history (see chapter 2.1.1), but its potential for future expansion is still unknown. I tackled the Colorado potato beetle's potential to invade north of its current range, that is, Finland. The species has been found in Finland for several years but has not yet established permanent populations.

Paper I examines the invasion history of the beetle in Europe by comparing the neutral genetic variation and gene flow of beetles collected from North America and different parts of Europe. This paper (I) is one of the few studies of the genetic consequences of continent-scale invasion of an insect pest. Species potential to change and adapt to new conditions can be studied via quantitative genetics by analysing the presence of additive genetic variance in important life-history traits (Hoffmann & Blows 1994, see also Lee et al. 2007). The absence of additive genetic variance in important life-history traits directly restricts species' range (Griffith & Watson 2006) and their potential to cope in the changing world (Hoffmann et al. 2003). To study the adaptive genetic variation among Colorado potato beetle populations, I conducted common garden rearing experiments with beetles collected from different parts of Europe. In paper II, I studied the adaptive genetic variation in life history traits of European beetle populations, particularly in development time, which is one important trait affecting the success of invasion of northern latitudes. As invasions are most likely to originate from marginal populations, paper III examined the performance of marginal populations in Russia and their potential invasion routes to Finland. Extreme abiotic factors might be important in restricting species' range (Gaston 2003, Lockwood et al. 2007). Night frosts during growing seasons are very common in northern regions and species invading these areas should have some pre-adaptation to deal with extreme changes in temperature. I therefore conducted an experiment on beetles' (egg and larvae) cold tolerance during the growing season, reported in paper IV.

As many invasive species are also pest species, human-induced selection is a very important factor contributing to invasion success. I studied the delayed and indirect effects of insecticide stress on the parental generation on their offspring's metabolic rate, lipid and water content, and size, reported in paper V. Taken together, my results help to delineate the Colorado potato beetle's invasion potential towards higher latitudes (e.g. Finland) as well as giving potential tools for a comprehensive management strategy.

2 MATERIALS AND METHODS

2.1 Study species

The Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera, Chrysomelidea) is a well-known invader and pest insect all over the northern hemisphere. The Colorado potato beetle uses species of the *Solanaceae* family, such as potato, tomato, tobacco and eggplant, as a host plant and causes severe yield reductions by defoliating the whole plant above ground level (Hare 1990). Its life cycle consists of an adult, egg, four larval stages, and a pupae stage (Weber 2003). Beetles emerge from diapause in the spring when the temperature is above 11°C and food plants are available (de Kort 1990). After females have gained enough energy (i.e. lipid), approximately one week after emergence (personal observation), they start laying eggs under the host plant leaves. Colorado potato beetles have four larval stages, all of which continuously eat the host plant leaves. A single larva can consume about 40 cm² of foliage in total (Weber 2003). Larvae of the fourth stage drop from the plant leaves to the ground and burrow themselves into the soil for pupation. The newly emerged adults may continue breeding after emergence or enter winter diapause after they have collected enough energy reserves for overwintering (de Kort 1990). Beetles overwinter as adults by burrowing into the soil (review in de Kort 1990) where diapause can be extended to several years (2-9 years) (Tauber & Tauber 2002). The length of the different life stages is strongly dependent on temperature (e.g. Ferro et al. 1985, Logan et al. 1985, Tauber et al. 1988, Hilbeck & Kennedy, 1998). At lower latitudes, there may even be three generations in one growing season but at higher latitudes there is typically only one.

The Colorado potato beetle is a successful invader and pest insect for several reasons. First, beetles have high fecundity as a female may lay up to 1300 eggs per growing season in the field (Jansson 1989, personal observation) and as many as 4000 eggs in laboratory conditions (Hare 1990). Secondly, they are very unpalatable (Hough-Goldstein et al. 1993a) and have an aposematic

colouration to advertise their unpalatability and thus do not have many natural enemies (Hough-Goldstein et al. 1993b). Furthermore, most of their parasitoids (e.g. the egg parasitoid *Edovum puttleri*) have not been able to follow the beetles to all the new areas the beetles have colonized, partly due to the cold climate conditions in Europe (Obrycki et al 1985). Thirdly, the Colorado potato beetle has been able to develop rapid resistance to a variety of insecticides (Casagrande 1987, Hare 1990). For example, the beetle developed resistance to permethrins during the second year of its use (Casagrande 1987). The Colorado potato beetle is naturally a poor flyer (Hare 1990), but is capable of long distance dispersal (~100 km) if helped by favourable weather conditions such as strong winds and storms (Wikteliuss 1982, Hare 1990). Human impact has contributed greatly to its rapid spread by transferring individuals from place to place by ship and train, and even on grocery lettuce (Finnish Food Safety Authority [EVIRA], Helsinki, personal communication).

2.1.1 The Colorado potato beetles invasion history

The Colorado potato beetle originated in Mexico where it used relatives of the wild potato, such as buffalo burr (*Solanum rostratum*), as a host plant (Casagrande 1987). Beetles were introduced to the U.S. in the 1860s when they began to use a cultivated potato as their host plant. Beetles spread rapidly throughout the U.S. within 100 years, expanding their distribution area by approximately 100 km a year (Johnson 1967). In 1922, Colorado potato beetles were accidentally introduced to Europe, to France, on a transport ship (Johnson 1967). Despite the probable small original population size, beetles spread almost throughout Europe, with the exception of the UK, Ireland and Scandinavia, in 50 years. The speed of invasion in Europe was on average 50 km per year (Johnson 1967).

The current northernmost beetle populations are in Russia between latitudes 61°N and 62°N (personal observation). During the summers of 2004, 2005 and 2006, we defined the beetles' distribution area around Lake Ladoga (see figures 1 & 2) establishing that the northernmost permanent population was found in Petroskoi (61°47'N) where the Colorado potato beetle has been a serious problem for the last 4 years and population densities have steadily increased year by year. For last two years Colorado potato beetles have been found near the Salmi region but beetle densities have remained low (a few individuals per field).

Beetles have been found in Finland in several different years but they have not yet been able to establish permanent populations. The worst year was 2002 when beetles were found in over 300 fields (Finnish Food Safety Authority, Helsinki). All the invaders have been successfully eradicated due to the effective control of the Finnish Food Safety Authority (EVIRA).

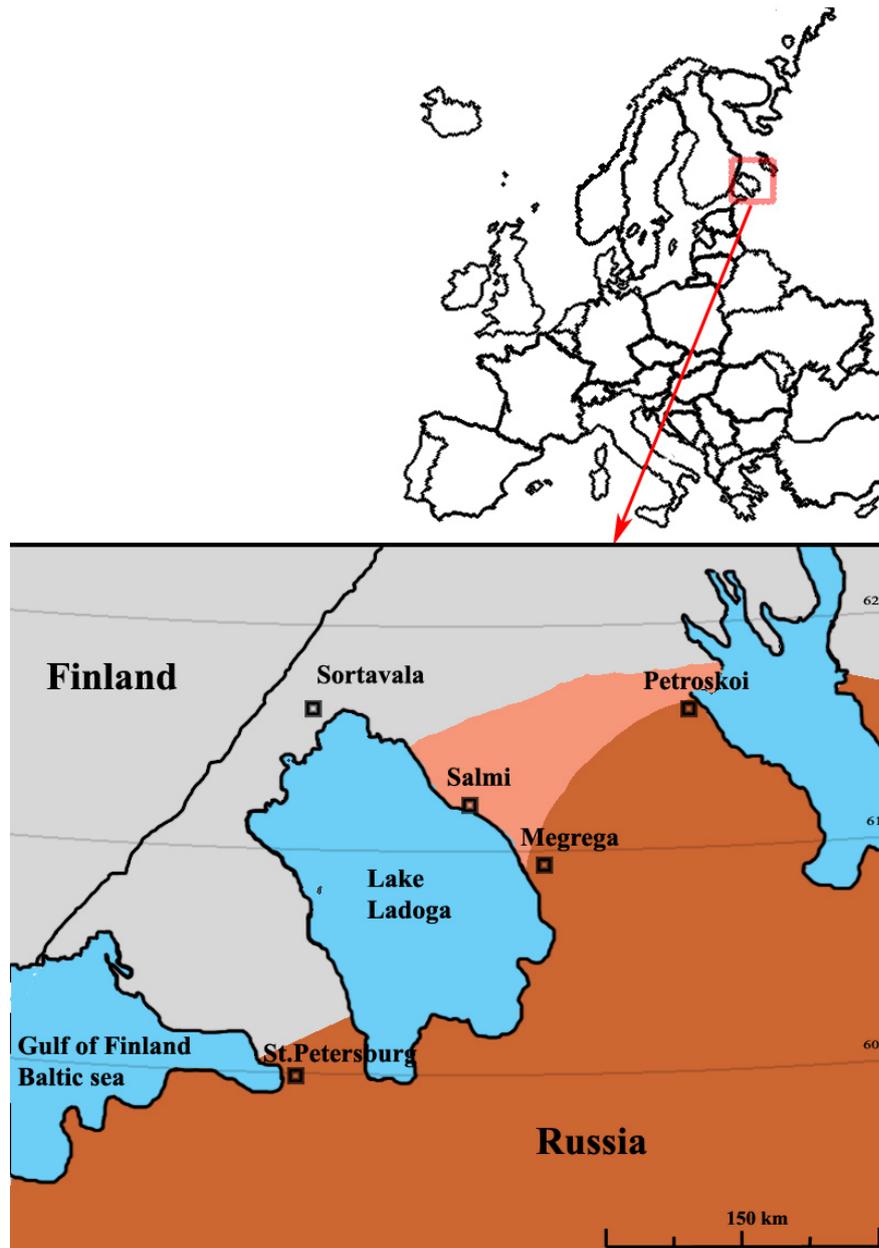


FIGURE 1 The current distribution area of the Colorado potato beetle in Russia. Areas marked with brown indicate permanent populations and areas marked with light brown indicate places where beetles have been found in the last two or three years, where population density is still very low. Observations were made in the period 2004-2006.



FIGURE 2 Collecting Colorado potato beetles in a potato field in Russia.

2.2 Analysing genetic variation (I, II)

By comparing geographically different populations we can gain important insights into population differentiation and structure and can assess organisms' potential for local adaptation. It is assumed that species need enough genetic variation for local adaptation which increases the individuals' fitness in new environments and thereby their chances of success (Gaston 2003, Sax et al. 2005).

The amplified fragment length polymorphism (AFLP) (Vos et al. 1995) and mtDNA techniques were used to ascertain the Colorado potato beetle's invasion history and to assess variability among populations in the North America and Europe (I). With the AFLP technique it is possible to rapidly screen the genetic diversity of DNA from a wide range of taxa and the technique is especially useful when assessing population structure and differentiation (review in Mueller & Wolfenbarger 1999). Moreover, genetic variation was analysed with mitochondrial DNA (mtDNA), which is inherited only from the mother and is therefore useful when tracing the direct genetic line between populations (Avise 1994). Analysis of mtDNA and AFLP markers was performed for beetles collected (during the summers of 2001 and 2002) from 13 populations (Fig. 3), five in North America [Colorado (n=36 beetles), Idaho (n=37), Kentucky (n=30), Minnesota (n=19) and New Brunswick (n=27)] and eight in Europe [Spain (n=37), France (n=38), northern and southern Italy (n=55 and n=18, respectively), Poland (n=51), Estonia (n=38), Russia (n=25) and Finland (n=32)]. The analyses were conducted in the DNA-laboratory at the University of Jyväskylä.

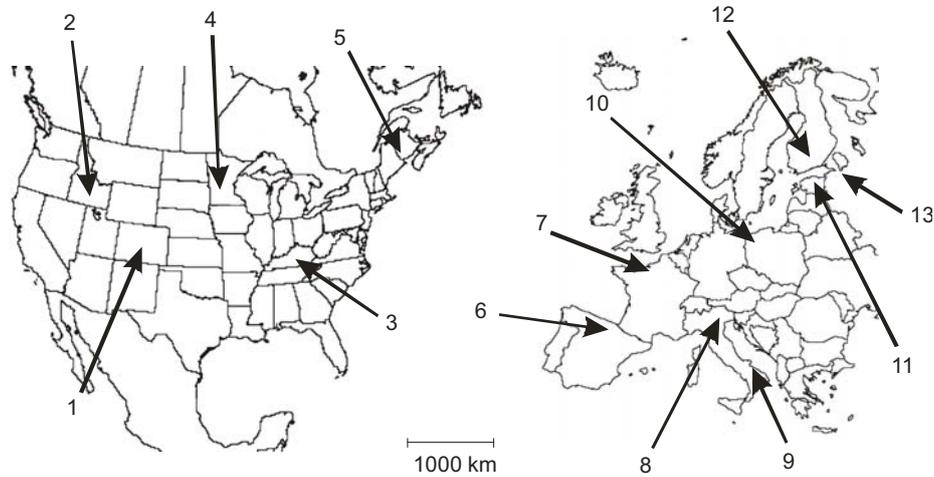


FIGURE 3 Locations of the North American and European populations of Colorado potato beetles. (1) Colorado, (2) Idaho, (3) Kentucky, (4) Minnesota, (5) New Brunswick, (6) Spain, (7) France, (8) Northern Italy, (9) Southern Italy, (10) Poland, (11) Estonia, (12) Finland, (13) Russia.

2.2.1 Genetic variation in life history traits (II)

The amount of adaptive genetic variation in life history traits was estimated by using quantitative genetic methods (II) (overview of the experimental design in Table 1). The beetles used in this experiment were descendants of field collected adults (collected in summer 2003) from Estonia (58°22'N), Poland (52°14'N), and Italy (41°13'N). A population was also collected from Russia and was maintained in a laboratory environment for three generations before the experiment to estimate the importance of maternal effects (Kawecki & Ebert 2004). This laboratory population was collected from the field in Russia (59°57'N [St. Petersburg]) in autumn 2001. The beetles overwintered in the laboratory at 5°C and were then reared to produce further generations under natural light and temperature conditions in a greenhouse in 2002. The actual experiment was begun in 2003. A full-sib design was used because the beetles from Estonia, Poland and Italy had mated in the field and the sires were unknown.

The experiment was carried out in two controlled environmental chambers (Type B1300, Weiss technic) housed at the University of Jyväskylä under a photoperiod of 4 hours dark, 2 hours dawn, 16 hours light and 2 hours dusk. One chamber was set to a variable regime in which the temperature was 13°C during the night, warming up to 20°C during the dawn, 20°C throughout day, declining back to 13°C during the dusk (average 17°C). The other chamber was set to a variable regime in which the temperature was 18°C during the night and 25°C during the day; warming up and cooling down during dawn and dusk respectively (average 23°C). The colder temperature regime reflects the conditions of a normal Scandinavian summer (June-July) while the higher

temperature regime reflects the conditions of an exceptionally warm summer (Drebs et al. 2002). A long-day photoperiod was chosen for both chambers because I was interested in studying the climate conditions of the northern range of the Colorado potato beetle.

Larvae were reared in Petri dishes lined with moist blotting paper (see Fig. 4) and fed daily with fresh potato leaves (variety *Van Gogh*, grown in a greenhouse). Larval-to-adult survival and development times were recorded (1st instar larva to adult). Adults were weighed ($\pm 1\text{mg}$) (AM100, Mettler) on the day of emergence and sexed.

I estimated the broad sense heritabilities and evolvabilities of development time and adult weight. The broad sense heritability ($h^2=V_G/V_P$) estimates the extent to which individual phenotypes are determined by the genotypes (Falconer & Mackay 1996) and varied between 0 and 1. I used the broad-sense estimates of heritability to avoid underestimation of the invasion potential of the beetles. The evolvability gives an estimate of the potential for selection to change the mean of a trait (Houle 1992). The term evolvability has multiple meanings (Pigliucci 2008) but is here defined as in Houle 1992: “ability of a population to respond to natural or artificial selection”. In other words, it gives an estimate of the species capacity to adapt to new conditions. The evolvability was calculated as $IA=(CV_A/100)^2$. Here, CV_A means the additive genetic coefficient of variance calculated as $CV_A=100 \times (\sqrt{V_A}/\text{trait mean})$, and the V_A is the additive genetic variance of a particular trait (Houle 1992). A value of $100 \times IA$ can be interpreted as a percentage trait change per generation as a response to selection (Hansen et al. 2003). It is important to note that the broad-sense heritability and evolvability are estimates, specific to a certain population and environment (Falconer & Mackay 1996). Therefore, comparing the evolvabilities and heritabilities with other species or populations is of limited utility (Falconer & Mackay 1996).

2.3 Measuring the fitness (III, IV, V)

2.3.1 Marginal populations performance (III)

As invasive pest species management is more effective at the earliest stage of invasion (Heikkilä & Peltola 2003, Genovesi 2005), assessment of the performance of marginal populations and their potential invasion routes to Scandinavia is essential for early detection and eradication. I assessed the performance of two populations inhabiting the northern margin of the Colorado potato beetle's European range: St. Petersburg [59°57'N] and Megrega [60°55'N], Russia, in terms of larval-to-adult survival, development time, adult size, sex ratio and overwintering mortality (overview of the experimental design in Table 1).

I collected egg clutches from potato fields (St. Petersburg $n=7$; Megrega $n=13$) and from females that laid eggs in the laboratory (St. Petersburg $n=7$;

Megrega n=17). Both field-collected and laboratory-produced egg clutches were kept at a constant 23°C until they hatched. Hatched larvae (St. Petersburg n=276 and Megrega n=523) were matched within family by their weight (± 0.02 mg) (Mettler Toledo, AT21 Comparator) and then divided between temperature regimes. Larvae were reared individually in Petri dishes lined with moist blotting paper and fed daily with fresh potato leaves (variety *Van Gogh*). Beetles were reared at two fluctuating temperature regimes with respective means of 17°C and 23°C (see experiment II for a more detailed description), which reflect the normal and warm Finnish summer temperatures. Larval-to-adult survival, sex and development times were recorded (1st instar larva to adult). Adults were weighed (± 1 mg) (AM100, Mettler) on the day of emergence and sexed.

To examine the overwintering success of adults, I recorded the beetles' overwintering survival. Beetles overwintered in soil-filled plastic jars that were kept in the dark at a constant temperature of 5°C. The soil was watered every second month to maintain adequate moisture. Overwintering survival was calculated by dividing the number of live beetles after overwintering by the number of beetles alive at the beginning of the wintering.

2.3.2 Cold tolerance (IV)

Night frosts are a common phenomenon in northern latitudes. Night frosts during the growing season may retard or even stop an invasion by the Colorado potato beetle if they have instant or delayed negative impact on its larval mortality. I tested whether the populations from the centre (Poland) and margins (Russia) of the European distribution range of the Colorado potato beetle differ in their responses to subzero exposure and low rearing temperature (overview of the experimental design in Table 1).

I used adult beetles, which were overwintered descendants of field-collected individuals from Russia (from four sites in a large area (150 - 200 km)) around latitude 59°N (Northern Russia) and Poland (one site) around latitude 54°N. Field-collected beetles were brought to the laboratory and their offspring were maintained at a constant 23°C and, after overwintering at 5°C, they were paired randomly within population and allowed to lay the eggs used in the experiment. Thus, our experimental larvae were the second laboratory generation minimizing possible maternal effects (Kawecki & Ebert 2004).

Larvae were maintained under a variable regime in which the temperature was 10°C during the 4 hours of darkness, warmed up to 15°C during the 2 hours of dawn, kept at 15°C throughout the 16 hours of light, and returned to 10°C during the 2 hours of dusk (average 13°C). The temperature reflects very cold summer conditions in Finland (Drebs et al. 2002, for example, summer 2008), conditions beyond beetles' current northern range.

To test whether larval cold tolerance varies with developmental stage (see Neven 2004), I exposed larvae of different ages (1 - 2, 9 - 10 and 19 - 20 days old) to -4°C during their night period. This subzero treatment is equivalent to night frosts, which occur during the summertime in northern latitudes. In central Finland (62°N, 25°E) and south-eastern Finland (61°N, 28°E),

respectively, there are on average 13.5 and 6.3 nights with below zero temperatures in the summer (based on over 40 years' climatological data from the Finnish Meteorological Institute; H. Pohjanoksa, personal communication). I measured beetles immediate larval mortality, development time, weight at emergence (± 1 mg) (AM100, Mettler), and larval-to-adult survival.

I also studied the cold tolerance of the eggs, verifying their hatchability after exposure to subzero temperature. In total, 48 egg clutches of 14 females from the marginal populations were divided into one control ($n = 12$) and three experimental groups of eggs of 2 – 3 ($n = 10$), 5 – 6 ($n = 15$), or 8 – 9 ($n = 11$) days old. This was done to determine whether susceptibility to -4°C varies with egg age. After exposure, eggs were maintained under the rearing conditions described above until their hatching success was recorded. Control eggs were maintained under the rearing conditions until the larvae hatched.

To examine the delayed impact of subzero treatment, adult overwintering success (of both control and experimental individuals) was recorded. Beetles overwintered in the dark at a constant 5°C from September to May. Overwintering mortality was calculated by dividing the number of dead beetles during wintering by the number of beetles alive at the beginning of the wintering. Overall survival was also calculated by dividing the number of survivors after overwintering by the number of larvae at the beginning of the experiment. I was thereby able to analyse the combined effects of low summer temperatures and cold shock on overwintering success.

2.3.3 Physiological traits and overwintering success (V)

The metabolic rate and lipid content of beetles before and after overwintering give important insights into an individual's performance and physiological condition. Overwintering success is usually associated with low metabolic rate and high lipid content, which is the main energy reserve during diapause (review in Hahn & Denlinger 2007). In temperate regions, it is important to have large energy reserves since the winter is long and cold. If an individual's metabolic rate is high and lipid reserves low at the start of the overwintering period, energy resources may be depleted too quickly decreasing an individual's ability to survive the winter (Hahn & Denlinger 2007). Since overwintering at the diapause stage is an energetically challenging life-history strategy, even mild environmental stress (e.g. insecticides) may cause injurious changes in an insect's physiological ability to withstand unfavourable overwintering conditions and dilute their potential to survive the winter (Carriere et al., 2001, Grewal et al. 2001). I assessed the influence of sub-lethal insecticide stress endured by the parental generation on their offspring's metabolic rates, lipid and water content, and size (overview of the experimental design in Table 1). I also assessed how possible negative changes in these traits affect offspring's overwintering success (V).

For the purposes of this experiment, a mixed population of Colorado potato beetles collected in 2003 from Finland (invading individuals found near the border with Russia), Russia ($59^{\circ}57'\text{N}$), Estonia ($58^{\circ}22'\text{N}$), Poland ($52^{\circ}14'\text{N}$),

and Italy (41°13'N) was reared on potted potato plants (Variety Van Gogh) in plastic cages (30 x 59 x 42 cm) placed in a greenhouse where beetles were allowed to mate randomly among different populations and lay eggs. An insecticide line was created by treating larvae (at approximately the 2nd larval instar) for three generations with synthetic pyrethroid insecticide (trade mark Decis) at a concentration of 0.0036 mg/l. The concentration was mild (the EC50 (median effective concentration) value for the water flea *Daphnia magna* is 0.0039 mg/l (Bayer Crop Science, 2008) to ensure non-lethal effects on beetles and prevent the evolution of insecticide resistance. A control line was maintained under similar conditions but without insecticide treatment. For the purposes of the actual experiment, females (insecticide: n = 8; control: n = 7) that mated randomly (within line) in plastic cages were transferred to Petri dishes and their egg clutches were collected daily. The offspring were not treated with insecticide but the two lines, offspring of insecticide treated beetles and of control beetles (later called control beetles), were maintained separately.

Larvae were reared at a fluctuating temperature regime (average 23°C) under a long-day photoperiod. Larvae were kept in groups of five individuals (within family) in small plastic boxes (0.5 L) lined with moist blotting paper and fed daily with fresh potato leaves (variety Van Gogh, grown in a greenhouse). Emerged adults were reared individually in Petri dishes at a constant temperature of 23°C under a long-day photoperiod (16L: 8D).

I estimated the metabolic rate of offspring from control and insecticide-treated parents using an open-flow respirometric system based on the Li-6252 CO₂ (LiCor, Lincoln, Nebraska, USA) analyser at 23°C. I weighed beetles (AM100, Mettler) and measured their metabolic rate in summer (at 5-days-old, control: n = 101, Insecticide: n = 99), and at the end of the overwintering period (overwintered 8 months, Control n = 60, Insecticide n = 59).

I also assessed beetles' lipid accumulation and depletion before and during overwintering. Lipid extractions (see detailed methodology in Östman 2005) from beetles were made in summer (at the age of 5 days) (Control females n = 10 and males n=9; Insecticide females n=10 and males n=10), in the middle of the diapause (at the age of 4 months) (Control females n=11 and males n=10; Insecticide females n=5 and males n=5), and at the end of the overwintering period (at the age of 8 months) (Control females n=35 and males n=29; Insecticide females n=37 and males n=22). I calculated the relative lipid content by dividing the lipid content by dry weight. Total water content was calculated for each beetle by subtracting the dry weight from the fresh weight and relative water content was calculated by dividing the total water content by dry weight. To examine whether the parental exposure to mild insecticide treatment affected offspring's overwintering success, I recorded the beetles' overwintering mortality. After emergence, adults were allowed to eat for 15 days and then moved to plastic pots (3.8 cm in diameter) containing peat for overwintering. Beetles were kept at the rearing temperature until they burrowed into the soil when they were transferred to the overwintering conditions. Beetles that did not burrow into the soil were kept for a maximum of 10 days at the rearing temperature and then moved to the overwintering conditions. Beetles

overwintered in the dark at a constant 5°C from August to March (8 months in total). To examine if insecticide alters beetle diapause behaviour, I visually recorded beetles digging behaviour (dug/did not dig into the peat) on alternate days. Beetles were weighed aged 15 days and after diapause. I was thereby able to calculate beetles' relative weight loss during overwintering ((weight at age 15 days (mg) - weight after diapause (mg))/ weight at the age of 15 days (mg)).

TABLE 1 An overview of the experimental designs

EXPERIMENT	II	III	IV	V
Experimental temperature	Fluctuating mean 17°C and 23°C	Fluctuating mean 17°C and 23°C	Fluctuating mean 15°C	Fluctuating mean 23°C
Population	Russia, Estonia, Poland and Italy	St.Petersburg and Megrega (Russia)	Poland and Russia	Mixed population (Finland, Russia, Estonia, Italy, Poland)
Treatment			Frost (-4°C) treatment for different age larvae and eggs	Parental generation under insecticide selection (three generations)
Traits measured	Larval-to adult survival, development time, adult weight	Larval-to adult survival, development time, adult weight	Larval-to adult survival, development time, adult weight	Larval-to adult survival, development time, adult weight, lipid content, metabolic rate, digging behavior
Overwintering success measured	No	Yes	Yes	Yes



FIGURE 4 Colorado potato beetles reared on Petri dishes.

3 RESULTS AND DISCUSSION

3.1 Genetic factors (I, II)

3.1.1 Genetic and phenotypic variability

Appraisal of neutral genetic variation can provide important information about species invasion routes and history as well as gene flow between different populations (Merilä et al. 1996, Björklund et al. 2007, Dlugosch & Parker 2008a). The presence of adaptive variability in life-history traits, in turn, can signify that a given species possesses the evolutionary capacity to respond rapidly to changing conditions, facilitating range expansion (Carroll & Dingle 1996, Garcia-Ramos & Rodriguez 2002, Lee 2002, Lee et al. 2007, but see Tsutsui et al. 2000). Therefore, I evaluated the potential of the Colorado potato beetle to invade north of its current range, by assessing its neutral genetic variability as well as its genetic and phenotypic variability in life history traits (i.e. development time, size and larval survival) (I, II).

The genetic analyses showed that the European populations of the Colorado potato beetle have lost genetic variability compared to North American populations (I). The AFLP markers and mtDNA showed a significant reduction in genetic diversity for all European populations whereas the populations in the central US showed the highest genetic variability of all populations. The European population was fixed for one mtDNA haplotype, which suggests that the European populations originate from a single successful invasion. Analysis was also performed on individuals (grouped as a population) found beyond the current range of the European populations, that is, in Finland. Genetic analysis showed that most of these Finnish beetles could be assigned to either the Russian or Finnish populations, indicating that the Russian populations were most likely the source of the Finnish invasion (see Fig. 1).

Although European populations showed significant reduction in neutral genetic variability compared to North American ones (I), the rearing experiment revealed genetic variation for development times, survival and

adult male size within populations (II). While the experimental set-up does not totally exclude possible maternal effects (Falconer & Mackay 1996), the results suggest the presence of additive genetic variance in European beetles. The fitness related traits of these populations have the potential to respond to selection (see Roff 1997) and evolve in novel environments (e.g. cooler climates) (Sakai et al. 2001, Lee 2002) which may facilitate beetles' invasion further north. However, the potential to adapt to new conditions does not necessarily signify that the beetle will invade further north, since there might be other factors restricting their spread such as scarcity of host plant, low overwintering success or short photoperiod.

There were no remarkable differences in life-history traits among Estonian, Polish and Italian populations (II), which might be explained by migration among them (I). Gene flow has been shown to hinder the evolution of local adaptation and, thus, reduce differences between ostensibly discrete populations (Hoffmann & Blows 1994, Kawecki & Ebert 2004). The Russian population, instead, differed from the other populations by having a significantly shorter development time and a higher survival at both temperatures. This may suggest that the short Russian summers could have selected fast-developing individuals, indicating adaptation to a short growing season (II).

Response to selection pressures might be restricted by the presence of trade offs between traits (Roff 1992). Fast-developing individuals became heavier than those that developed slowly indicating that fast development time does not constrain size (II). This will most likely improve beetles' chances of successfully invading and establishing populations in the north, since beetles can accelerate their development without negative costs in terms of size. Large body size and fast development time may contribute to diapause preparation and overwintering survival, especially for northern populations such as those in Russia and Estonia. As time available for growth in the north is limited and winter comes early, it might be beneficial to reach a large body size in a short time to cope with the adverse conditions. Furthermore, size may contribute to beetles' ability to dig into the soil for overwintering as larger beetles are able to burrow deeper than are smaller ones and thereby gain more protection from frost during diapause (Noronha & Cloutier 1998). The lack of trade off between these traits is, however, most likely the result of strong selection by insecticides (during the early larval stages) as well as the harvesting of potatoes twice in one season (limiting the food resource). These factors both favour fast developing individuals, which can reach large larval or adult stage early and thus can better tolerate insecticide exposure or, alternatively, reach the adult stage before the host plant is harvested.

High heritability and evolvability are important for invasive species because they express the ability of populations to respond to selection (Houle 1992, Roff 1997). European beetles exhibited significant and relatively high heritability for development time and adult size, indicating a high level of additive genetic variation (II). This suggests that beetles possess the capacity to respond to selection by short growing season and, thus, to accelerate their

development which is vital for successful colonization of higher latitudes. The evolvabilities calculated in this study predict that it would take the Russian population approximately 6 generations at 17°C and 7 generations at 23°C to accelerate development time by one day. Adult weight showed a high capacity to change across generations. Percentage differences ranged from 0.179 to 2.142% per generation. However, my findings of high heritability and evolvability should be interpreted cautiously since full-sib heritability is known to be inflated because it also includes maternal effects (Falconer & Mckay 1996). The heritability estimates thus represent upper limit values (Roff 1997). Nevertheless, in assessing invasion potential, it is safer to use upper limit estimates to avoid under-estimation of invasion risk. In any case, these results suggest that the Colorado potato beetle still has potential to respond to selection and to evolve, indicating that genetic factors do not seem to constrain the beetles' invasion to higher latitudes.

3.2 Abiotic factors (II, III, IV, V)

3.2.1 Temperature

Temperature may directly limit species' distribution by killing them or indirectly through interaction with other factors such as food supply (e.g. Zaslavski 1988, Chown & Nicolson 2004). Temperature may have strong impact on the life history traits of insects, such as development, reproduction and diapause (Ferro et al. 1985, Tauber et al. 1988, Noronha & Cloutier 1998, Chown & Nicolson 2004). It has been shown that Colorado potato beetles optimum temperature for development is 28°C (Ferro 1985), thus, the summer temperatures in Finland might be too cold for beetles and they would not be able to develop from egg to adult at normal summer temperatures. However Ferro's (1985) experiments were conducted with beetles originating from North American populations and the results might not be directly applicable to European populations. Therefore I assessed whether the summer temperature conditions existing north of the beetles' current range restrict their invasion further north (II, III, IV).

As expected, temperature strongly affected all the life history traits studied (II, III and IV). Development times were approximately 12-14 days longer at the colder than at the warmer temperature regime (II, III). Furthermore, larval survival was higher (about 20 - 30% depending on population) at the warmer temperature (II). Surprisingly, I did not find any differences in larval survival between temperatures treatments, reported in paper III.

The phenomenon of either increasing or decreasing body size with latitude has been observed in insects (Blanckenhorn & Demont 2004, Chown & Nicolson 2004). In my experiments, there were no differences in adult size among populations (II); neither sex showed a latitudinal cline (II). My research

offered no clear evidence for a latitudinal gradient in body size in the Colorado potato beetle. Results from the rearing experiment were similarly inconsistent. Beetles grew larger at the colder than at the warmer rearing temperature (all populations, II) or other way around (III) or no differences were found (Megrega population).

A low rearing temperature (mean 12–13°C) dramatically affected larval survival, inducing about 95% mortality (IV). Such high mortality suggests that this is near to the threshold temperature for Colorado potato beetles' development (see also Ferro et al. 1985). Since mortality was high, the Colorado potato beetle would require a very large propagule size to invade, even though the beetle's high fecundity (see Jansson 1989, Hare 1990, personal observation) may compensate to some degree for the high larval-to-adult mortality. Still, it is important to note that 1.1% of the beetles survived to adulthood making it at least theoretically possible for beetles to establish populations under these harsh conditions. There have been cases when even the invasion of a single mated female resulted in successful establishment (Grevstad 1999, Memmott et al. 2005). Short exposure to sub-zero temperature (frost) did not have any effect on egg hatchability, larval survival, development or adult size. Thus, frost during the growing season is not, *per se*, an important limiting factor for Colorado potato beetles' northern range.

Taken together, the results showed that the beetle is able to develop and survive at normal summer temperatures beyond its current northern range (II, III) and even at colder temperatures (IV), indicating that normal summer temperatures do not restrict beetles' northward invasion and, particularly, their movement toward Finland.

3.2.2 Overwintering (III, IV, V)

Valosaari et al. (2008) have shown in their model that overwintering mortality is one of the important limiting factors for the Colorado potato beetles' northward invasion. I studied beetles' overwintering success in experiments reported in papers III, IV and V. My results demonstrate that the beetles are able to overwinter for a prolonged diapause period of about 8 months, which corresponds to the winter period in Finland. In fact, beetles are able to survive in prolonged diapause for several years (2–9 years) (Tauber & Tauber 2002). Prolonged diapause could be advantageous especially in higher latitudes, since beetles could continue their diapause if the summer conditions were unfavourable to them and breed only during favourable summer conditions.

Large individuals often have high lipid content which is a key element in withstanding sometimes prolonged starvation and harsh winter conditions during the diapause period (Hahn & Denlinger, 2007; Matsuo, 2006). Hiiesaar et al. (2006) have shown that Colorado potato beetles weighing less than 100 mg were not able to survive overwinter in Estonia. Indeed, overwintering survival was affected by the size of the individuals in the experiments of this thesis (III, IV). The average weight of the beetles that survived over winter was 114.6 (± 19.6) mg ($n=194$) and of beetles that died during overwintering 109.7 (± 18.7)

mg (n=212) (paper III). However, size had no effect on the overwintering survival of the offspring of insecticide treated and control beetles in the insecticide experiment (V). But beetles overwintering behaviour was affected by cross-generational effects as the progeny of insecticide treated parents showed less burrowing activity than control beetles, which was associated with increased overwintering mortality.

The overwintering temperature was 5°C in all experiments which was a little higher than overwintering temperatures would naturally be in Finland. The soil temperature in Finland varies greatly depending on the snow coverage and the air temperature (Yli-Halla & Mokma 1998). For example, in winter 2001/2002 the soil temperature at the depth of 50 cm in December in southern Finland was 1.2°C and at the depth of 90 cm 3.0°C (Hänninen et al. 2005). In nature beetles burrow to a depth of approximately 60cm (de Kort 1990) but the digging depth is dependent on temperature, soil conditions and beetles' size (Noronha & Cloutier 1998). In 2004, a few individuals were found in Finnish fields extremely early in the spring suggesting that those individuals had successfully overwintered in Finland (Finnish Food Safety Authority, Helsinki, personal communication). This indicates that, at least during mild winters, overwintering is not a restricting factor for successful invasion. Hiiesaar et al. (2006) have shown in field experiments conducted in Estonia that the Colorado potato beetle is not able to overwinter if there is a long period of extreme cold (-30°C) during winter (mortality 88 - 100%), but during a mild winter the mortality varied only between 12 and 20% depending on soil type. However, further experimentation is needed to fully understand the Colorado potato beetles' potential to overwinter in Finland. It is not possible to conduct a field experiment in Finland because of the quarantine status of this pest insect.

3.2.3 Insecticides (V)

It is well known that insecticides have negative impact on the behaviour and fitness of many insects (e.g., Grewal et al. 2001, Desneux et al. 2006, Byrne & Toscano 2007, Wang et al. 2008). However, less is known about the cross-generational effects of sub-lethal doses of insecticides on the physiology and behaviour of the progeny of insecticide-treated parents (but see Bauce et al. 2006). I assessed the influence of insecticide stress on the parental generation on the fitness of their offspring (V). A parallel situation in nature would be that the parental generation is exposed to insecticides and thereafter invades new areas where insecticides are not used. Thus, it is highly relevant and important to determine the effects of insecticides on offspring's fitness and overwintering success, that is, beyond the exposed generation.

I found that beetles descended from insecticide-treated parents were smaller, had a higher metabolic rate at the age of 5 days and lower water content. Furthermore, a higher proportion of offspring of the insecticide-treated beetles overwintered on the soil surface, which potentially caused higher overwintering mortality. On the other hand, offspring of insecticide-treated beetles had higher fat content than the progeny of non-treated beetles, which

could be a tremendous advantage for overwintering success and thus contribute to subsequent reproduction success. Higher reproductive success, in turn, might lead to higher population densities. These results demonstrated that insecticides may have fitness costs but may also have beneficial effects even beyond the generation exposed to the insecticide. Taken together, the cross-generational effects of insecticides may influence the fitness of subsequent populations by altering beetles' physiology, behaviour and life history traits which are important for survival and reproduction, thus causing evolutionary changes even at the population level (e.g. Magiafoglou & Hoffmann 2003, review in Räsänen & Kruuk 2007).

3.3 Potential invasion routes (III)

One key to effective management and prevention of invading species is to identify potential source populations at the current range margins of the species. This can be done by assessing their performance in the environmental conditions beyond their current range (e.g. Gilman 2006, Lyytinen et al. 2008, II). Permanent populations at the edge of the Colorado potato beetles' distribution area are in Russia near latitude 61°N (Fig. 1). I studied the performance of two populations on this northern margin: St. Petersburg and Megrega (III). The St. Petersburg population was established at the beginning of the 1980s, but has not been able to invade to Finland through the Karelian Isthmus. The Megrega population was established quite recently, about 5 years ago (personal observation), and the beetles have since expanded their distribution to the eastside of Lake Ladoga (see Fig 1).

Both populations were able to develop in temperatures reflecting those of a normal Finnish summer and with a prolonged overwintering period (8 months) at 5°C. The Megrega population had a significantly faster development time, indicating some evidence of rapid adaptation to local conditions, which may increase beetles' potential to invade Finland. On the other hand, beetles from the Megrega population were smaller than those of the St. Petersburg population. Neither the larval-to-adult nor overwintering survival showed any differences between the two populations.

Taken together, both populations have the potential to invade north of their current range as they were able to develop and survive at temperatures lower than in their current range. Moreover, the Megrega population showed some evidence of adaptation to a short growing season (i.e. fast development time), which might improve their potential to spread to higher latitudes. Thus, one possible invasion route towards Finland might be from the eastside of Lake Ladoga, which is not the shortest distance between beetle populations and Finnish potato fields (Fig. 1). However, there might be other factors restricting beetles' invasion further north such as food plant distribution. It is possible that a scarcity of potato fields in the Karelian Isthmus, on the eastside of Lake

Ladoga and in eastern Finland may slow down the beetles' northward progression.

4 CONCLUSIONS AND FUTURE GOALS

Assessing species invasion potential to new areas gives us tools to recognize potential invaders and favourable habitats for invasion. Such knowledge is also likely to aid in the prevention and management of these species after their colonization (Genovesi 2005). This is particularly important as most of the literature on invasions biology deals with successful invaders (Whitney & Gabler 2008). To understand species' invasion potential, we need to consider both the ecological and evolutionary aspects (Lee 2002, Gaston 2003). This means that we should know whether a species is able or unable to survive and reproduce in a new environment as well as whether the species can further adapt to the new conditions (Lee et al. 2007).

I assessed the potential of a well-known invader, the Colorado potato beetle, to expand north of its current range in Europe. Comparison of neutral genetic variability among populations revealed that European beetles underwent a founder effect and/or bottleneck when they reached Europe from United States, which markedly reduced their neutral genetic variation (I). This is a common finding in invasive species (Dlugoush & Parker 2008). However, neutral genetic variability can be a poor measure of the adaptive potential of populations (Dlugoush & Parker 2008). I showed that European Colorado potato beetles still have adaptive genetic variation in their life history traits, especially for development time which showed high heritability (II). The Russian population differed from the other European populations studied by having a significantly shorter development time and a higher survival rate at both a higher and a lower temperature when the beetle populations were reared on two fluctuating temperature regimes. This may suggest that short summers in Russia could have selected fast-developing individuals, indicating adaptation to short growing season (II). Given that they are the most likely source for an invasion of Finland (I), summer temperatures beyond the current range are unlikely to restrict invasion. Furthermore the marginal populations in Russia showed some evidence of rapid local adaptation, as individuals of the Megrega population developed significantly faster than those of the St. Petersburg population (III). These results indicate that European Colorado potato beetles

have potential for further evolution in life history traits, which might improve their colonization success.

Rearing individuals under average conditions beyond their current range is one way of testing their invasion potential. On the other hand, establishment in a given area might be restricted by thermal extremes more than by the average temperature (Inouye 2000, Tesar & Scriber 2003). In this thesis, I have shown that the neither normal Finnish summer temperature (II) nor harsh conditions (frosts) during the growing season (IV) limits the Colorado potato beetles' potential to expand its range. Beetles were able to develop from larva to adulthood at normal Finnish summer temperatures and exposure to subzero temperature during the larval stage did not increase mortality. Even though very cold growing temperatures incur high mortality, 1.1% of beetles could survive to the following growing season. Furthermore, beetles were able to survive a prolonged overwintering period (8 months) at 5°C suggesting that, at least during mild winters, overwintering beyond its current range is not a problem for this species. However, further experiments are still needed to confirm whether the Colorado potato beetle is able to overwinter in Finland during cold winters and what kind of physiological tolerance is needed in terms of survival under harsh winter conditions. Studies investigating overwintering stress in combination with another abiotic stress factor (as shown in paper V), in particular, may provide important information for the prevention of Colorado potato beetles' expansion and for its future management.

Human-induced selection by pesticides probably restricts the invasion of many pest species initially. Selection by pesticides may help to eradicate small invading populations since such populations may not be able to respond to or tolerate the insecticides due to the low genetic variation in their fitness related traits. The sub-lethal insecticide exposure of parental generation caused fitness costs in their (unexposed) offspring, such as smaller size and higher metabolic rate, and altered the offspring's diapause behaviour (V). These costs may negatively affect overwintering success. Surprisingly, the insecticides caused beneficial effects as the offspring of the insecticide-treated beetles had higher fat content than did control beetles. Large fat reserves are a prerequisite for successful overwintering, especially during long and harsh winters. Thus, insecticides may, in fact, improve overwintering success.

Genetic markers inferred that the source of the current Finnish invasions is most likely Russian populations (I). Since the Colorado potato beetle is not a quarantine pest in Russia, the beetle populations near the Finnish border may spread freely from place to place. Thus, during favourable summers Russian population sizes may increase rapidly which may also increase the propagule size of the invading populations. Pest management by insecticide in Russia is also largely dependent on individual professional farmers. Therefore, it is not necessarily undertaken according to the instructions of the manufactures (personal observation). The potential invasion routes towards Finland are through the Karelian Isthmus or from the eastside of Lake Ladoga (Fig. 1). However, the scarcity of potato fields in these areas, and in eastern Finland,

may retard the beetles' invasion and their further colonization success (Valosaari et al. 2008).

Taken together, my results suggest that Colorado potato beetles possess the ability to adapt to the climatic conditions existing north of their current range in Europe and thus have the potential to expand their range to Finland. Even though the beetle has not yet been able to colonise Finland, there is still a need for continuous surveillance by the Finnish Food Safety Authority (EVIRA) as early detection and eradication is still the most successful management strategy (Heikkilä & Peltola 2003). The eradication of beetles by insecticide has been successful in Finland so far (EVIRA), which is one reason why the beetle has yet not established permanent populations in Finland. However, the use of insecticides is a double-edged sword since it may have both positive and negative impact on beetles' fitness (V) and may hasten the evolution of insecticide resistance making the species more persistent (Mallet 1989). Thus, if we want to continue successful eradication of this pest, we should better understand the possible risks of insecticides.

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YHTEENVETO (RÉSUMÉ IN FINNISH)

Ekologisten ja geneettisten tekijöiden vaikutus koloradonkuoriaisen (*Leptinotarsa decemlineata*) leviämismenestykseen

Lajien levinneisyyteen ja runsauteen vaikuttavien tekijöiden tutkiminen on keskeinen osa ekologiaa. Perinteisesti on tutkittu abioottisten ja bioottisten tekijöiden vaikutusta lajien levinneisyyteen huomioimatta lajien kykyä sopeutua muuttuviin oloihin. Jotta leviäminen olisi mahdollista, lajilla pitäisi olla riittävästi geneettistä vaihtelua leviämisen kannalta tärkeissä elinkierto- ja elinympäristöissä. Tutkimalla lajien leviämistä rajoittavia ekologisia ja evolutiivisia tekijöitä voidaan selvittää niiden potentiaalia levitä uusille alueille.

Vieraslajeiksi sanotaan lajeja, jotka ihmisen toiminnan yhteydessä, tarkoituksellisesti tai huomaamatta, ovat siirtyneet maantieteelliseltä alueelta toiselle. Vieraslajit ovat maailman luonnon monimuotoisuuden toiseksi suurin uhka heti elinympäristöjen tuhoutumisen jälkeen. Ekologisten vaikutusten ohella vieraslajit voivat aiheuttaa merkittävää taloudellista haittaa, tästä hyvänä esimerkkinä ovat viljelykasvien tuhohyönteiset. Vieraslajien torjuntaan ja eliminointiin käytetäänkin vuosittain huomattavia rahasummia. Tästä syystä olisi tärkeää pystyä ennustamaan, mitkä lajit luultavimmin leviävät millekin alueelle ja miten ne vaikuttavat sen ympäristön eliöyhteisöön.

Koloradonkuoriainen (*Leptinotarsa decemlineata*) on perunan merkittävin tuhohyönteinen maailmanlaajuisesti, ja viime kesät ovat osoittaneet sen olevan leviämässä myös Suomeen. Alun perin kuoriainen on lähtöisin Meksikosta, josta se levisi Yhdysvaltoihin ja sieltä edelleen ihmisen mukana Eurooppaan 1900-luvun alkupuolella. Nykyisin koloradonkuoriainen on hyvin yleinen tuhohyönteinen Euroopassa lukuun ottamatta Brittein saaria ja Skandinaavia. Lähimmät Suomea ympäröivät koloradonkuoriaispopulaatiot ovat Venäjällä ja Virossa. Suomesta näitä tuhohyönteisiä on löydetty vuodesta 2002 (havaintoja 324 perunapellolta) alkaen lähes jokaisena kesänä.

Koloradonkuoriainen on Suomessa levinneisyytensä ääri rajoilla, sen vuoksi on tärkeää selvittää koloradonkuoriaisten kykyä selviytyä alhaisissa lämpötiloissa ja niiden kykyä sopeutua Suomen viileään ilmastoon. Hyönteisten kehitysnopeus on voimakkaasti lämpötilasta riippuvainen. Esimerkiksi keskilämpötilan ollessa liian alhainen koloradonkuoriaisten kehittyminen häiriintyy ja ne kuolevat. Tämä voisi osaltaan selittää, miksi koloradonkuoriaiset eivät ole levinneet pohjoiseen kuten Skandinaaviaan; toisin sanoen pohjoisessa kuoriaiset eivät ehtisi kehittyä riittävästi ennen talven tuloa ja ilmasto olisi liian kylmä talvehtimiselle. Lajien leviämiseen vaikuttaa kuitenkin myös niiden kyky sopeutua uuteen ympäristöön. Jos koloradonkuoriaisen elinkierto- ja elinympäristöissä esiintyy geneettistä vaihtelua, saattaa kuoriaisen sopeutuminen alempiin lämpötiloihin olla mahdollista, joka edesauttaisi niiden leviämistä Suomeen.

Väitöskirjassani tutkin koloradonkuoriaisen leviämiseen vaikuttavia geneettisiä ja ekologisia tekijöitä, sekä yleisesti yksilöiden kykyä sopeutua uuteen ympäristöön. Keskityin erityisesti tarkastelemaan koloradonkuoriaisten popu-

laatorakennetta, maantieteellistä vaihtelua elinkierto- ja abiottisten tekijöiden kuten lämpötilan ja torjunta-aineen vaikutusta kuoriaisen selviytymiseen.

Tutkimukseni osoitti, että eurooppalaiset koloradonkuoriaiset ovat menettäneet neutraalista geneettistä vaihtelua levitessään Eurooppaan johtuen pullonkaula- ja/tai perustajavaikutuksesta. Tästä huolimatta kuoriaisilta löytyy geneettistä vaihtelua elinkierto- ja abiottisten tekijöiden vaikutuksesta, kuten kehitysajassa ja koossa, jonka avulla kuoriaiset voisivat sopeutua entistä paremmin uusiin olosuhteisiin, esimerkiksi alhaiseen lämpötilaan. Tämän perusteella geneettiset tekijät eivät rajoita kuoriaisen leviämistä yhä pohjoisemmaksi kuten Suomeen.

Koloradonkuoriaiset pystyivät kehittymään ja selviytymään Suomen normaaleissa kesäolosuhteissa (keskilämpötila 17°C). Myöskään hallayöt (3 tuntia/4°C) eivät vaikuttaneet kuoriaisen toukkien kehitykseen tai kuolleisuuteen. Alhaisessa kesälämpötilassa (13°C) kasvatettujen koloradonkuoriaistoukkien kuolleisuus oli kuitenkin erittäin korkea noin 90%, joten erittäin koleina kesinä kuoriaisen elinmahdollisuudet Suomessa ovat vähäiset. On kuitenkin hyvä muistaa, että koloradonkuoriainen kykenee lisääntymään erittäin tehokkaasti. Yksi emo saattaa munia kasvukauden aikana jopa 1 300 munaa, mikä saattaisi kompensoida korkeaa kuolleisuutta. Tulosten perusteella normaalit kesälämpötilat eivätkä hallayöt estä kuoriaisen leviämistä Suomeen, mutta alhaiset kesälämpötilat (13°C) saattavat jossain määrin toimia rajoittavana tekijänä.

Viime vuosien koloradonkuoriaisinvaasiot on Suomessa onnistuttu estämään torjunta-aineilla. Vaikka torjunta-aineilla saadaan usein kontrolloitua tehokkaasti tuhohyönteisiä, on niiden käytössä kuitenkin myös useita riskejä. Torjunta-aineiden liiallinen käyttö voi johtaa torjunta-aineresistenssin yleistymiseen tai aiheuttaa positiivisia muutoksia tuhohyönteisten käyttäytymisessä ja fysiologiassa (esim. parantaa lisääntymiskykyä). Viidennessä osatutkimuksessa tarkastelin torjunta-aineilla käsiteltyjen sekä käsittelemättömien kontrolli-emojen jälkeläisiä. Kuoriaiset, joiden emot oli käsitelty torjunta-aineella, olivat pienempiä, niillä oli vilkkaampi metabolia ja alhaisempi suhteellinen vesipitoisuus. Lisäksi suurempi osa torjunta-aineilla käsiteltyjen emojen jälkeläisistä jäi talvehtimaan multakerroksen pinnalle, joka oli yhteydessä korkeampaan talvikuolleisuuteen. Torjunta-aine aiheutti kuitenkin myös positiivisia vaikutuksia kuoriaisten fysiologiaan, sillä torjunta-aine käsiteltyjen yksilöiden jälkeläisillä oli suhteellisesti enemmän rasvaa kuin kontrollikuoriaisilla. Korkea suhteellinen rasvan määrä on eduksi talvehtimisen aikana, etenkin jos talvi on pitkä ja kylmä kuten Suomessa.

Populaatiogeneettiset analyysit osoittivat, että viime vuosina Suomesta löydetyt kuoriaiset ovat lähtöisin Venäjältä. Venäjällä tehtyjen maastokartoitusten (vuosina 2004 - 2006) perusteella havaittiin, että pohjoisimmat koloradonkuoriaispopulaatiot sijaitsevat Petroskoissa. Yksi todennäköinen leviämisreitti Suomeen saattaakin olla Laatokan itäpuolelta, jossa koloradonkuoriainen on viime vuosina vähitellen levittäytynyt yhä pohjoisemmaksi. Sen vuoksi Suomessa koloradonkuoriaisen tarkkailua tulisi edelleen jatkaa erityisesti Venäjän rajan läheisyydessä.

Tutkimukseni osoitti, että koloradonkuoriaisella on potentiaalia levitä Suomeen pysyvästi, sillä geneettiset tekijät eivätkä Suomen kesälämpötilat rajoita koloradonkuoriaisen leviämistä pohjoiseen. Kuoriaisen potentiaali levitä pohjoiseen ei kuitenkaan suoraan merkitse sitä, että kuoriainen leviäisi lähivuosina pysyvästi Suomeen. Myös muutkin tekijät, kuten perunanviljelyalueiden sijainti tai talvehtimismenestys, saattaa rajoittaa kuoriaisen leviämistä Suomeen. Laajimmat perunanviljelyalueet sijaitsevat Länsi-Suomessa, kun taas lähellä Venäjän rajaa perunaa viljellään vain paikoitellen. Myös Venäjän puolella perunanviljelyalueet Karjalan Kannaksella ja Laatokan itäpuolella ovat harvassa, mikä voisi olla syy siihen miksi kuoriainen ei ole vielä levinyt Suomeen. Myös talvehtimismenestys on yksi tärkeimmistä tekijöistä, jotka määrittävät koloradonkuoriaisen leviämisaluetta. On mahdollista, että kuoriainen ei kykene talvehtimaan menestyksekkäästi Suomen talviolosuhteissa, ja siten estäisi kuoriaisen pysyvän leviämisen Suomeen. Toisaalta koloradonkuoriainen kykenee talvehtimaan Venäjällä Petroskoin korkeudella, jossa talviolosuhteet saattavat olla jopa kylmemmät kuin Etelä-Suomessa. Tämä viittaa siihen, etteivät kylmät talviolotkaan välttämättä rajoita koloradonkuoriaisen leviämistä Suomeen.

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