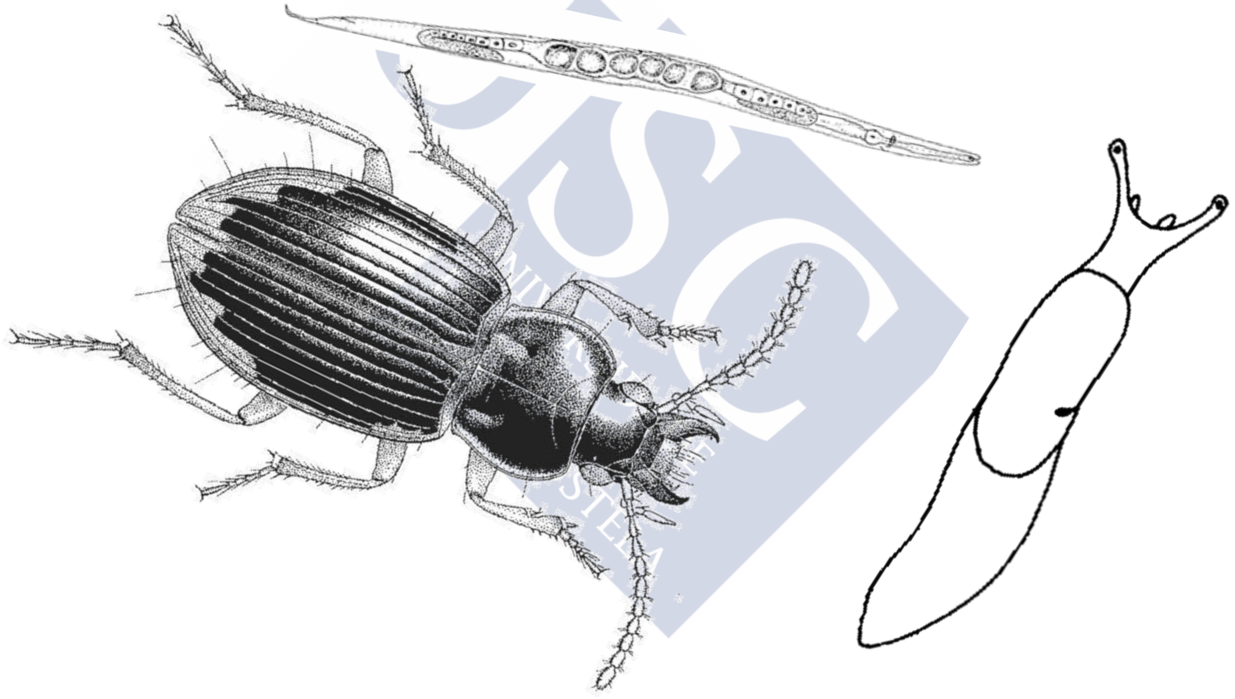
TESE DE DOUTORAMENTO - PhD THESIS

MODALIDADE DE COMPENDIO DE ARTIGOS – COMPILATION OF PUBLICATIONS

EFFECTS OF CLIMATE CHANGE ON TERRESTRIAL SLUGS AND THEIR PREDATORS

Heba-T-Alla El-Danasoury



PROGRAMA DE DOUTORAMENTO EN BIODIVERSIDADE E
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FACULTADE DE BIOLOXÍA

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Javier Iglesias Piñeiro, Profesor Titular de Universidade do Departamento de Zooloxía, Xenética e Antropoloxía Física, área de Zooloxía, como director da tese titulada ***“Effects of climate change on terrestrial slugs and their predators”*** pola presente,

DECLARO:

que a tese de doutoramento presentada por ***Dona Heba-T-Alla El-Danasoury*** é idónea para ser presentada, de acordo co artigo 41 do Regulamento de Estudos de Doutoramento, pola modalidade de compendio de ARTIGOS, nos que o doutorando tivo participación no peso da investigación e a súa contribución foi decisiva para levar a cabo este traballo. E que está en coñecemento dos coautores, tanto doutores como non doutores, participantes nos artigos, que ningún dos traballos reunidos nesta tese serán presentados por ningún deles noutra tese de Doutoramento, o que asino baixo a miña responsabilidade.

Santiago de Compostela, a 15 de Febreiro de 2017.

Javier Iglesias Piñeiro


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This PhD thesis is based on the following papers

Esta tesis de doctorado está basada en los siguientes artículos

Esta tese de doutoramento está baseada nos seguintes artigos

El-Danasoury H., Iglesias-Piñeiro J., Córdoba M. (2016) The effect of climate manipulations on the herbivory of the pest slug *Deroceras reticulatum* (Müller, 1774) (Pulmonata: Agriolimacidae). *International Journal of Biometeorology*, **60**, 1501-1507. doi:10.1007/s00484-016-1140-0.

El-Danasoury H., Cerecedo C., Córdoba M., Iglesias-Piñeiro J. (2016) Predation by the carabid beetle *Harpalus rufipes* on the pest slug *Deroceras reticulatum* in the laboratory. *Annals of Applied Biology*, doi:10.1111/aab12337.

El-Danasoury H., Iglesias-Piñeiro J. (2017) Predation by polyphagous carabid beetles on eggs of the pest slug *Deroceras reticulatum*: effect of temperature and potential implications of climate change.
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To my Father and Mother

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GENERAL INTRODUCTION


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GENERAL INTRODUCTION

TERRESTRIAL GASTROPODS AS AGRICULTURAL PESTS

With approximately 35,000 current species, gastropods can be considered one of the most successful and diverse groups in terrestrial ecosystems (Barker, 2001). In natural communities, terrestrial gastropods act mainly as primary consumers, although their quantitative importance in this regard is generally considered small (Curry, 1994). However, the strong selective pressure that they exert on plants, profoundly affects the morphology, phenology and defensive systems of the species they consume (Rathcke, 1985), as well as the production, composition and diversity of plant communities (Dirzo and Harper, 1982, Cottam, 1986, Oliveira Silva, 1992; Speiser, 2001). In addition, terrestrial gastropods physically and chemically contribute to the recycling of organic matter (Mason, 1974, Chatfield, 1976; Jennings and Barkham, 1976, 1979; Richter, 1979; Curry, 1994; Theenhaus and Scheu, 1996). It has also been pointed out that their faeces and mucus may contribute to the structuring of soil (Newell, 1967).

Terrestrial gastropods have always been closely related to humans, not only because they have been used as food since prehistory, but also due to aspects related to religion, culture or economics (Godan, 1999). The consumption of terrestrial gastropods by humans has focused almost exclusively on snails, while references to slug consumption are anecdotal and often related to folk medicine (South, 1992; Godan, 1999). At present, commercial snail farming are widespread in many countries around the world (Iglesias and Castillejo, 1997).

The ability of snails and slugs to store contaminants in their tissues makes them excellent indicators of contamination, especially for heavy metals (Martin and Coughtrey, 1982; Dallinger *et al.*, 2001). Also, terrestrial gastropods are frequently used as laboratory animals and are used as models in studies of neurophysiology, physiology of the circulatory system, behavioral ecology, or population genetics (Godan, 1999; Barker, 2001).

As negative aspects, terrestrial gastropods can cause severe damage to crops and act as vectors of transmission of plant pathogens (viruses, fungi) and intermediary hosts of parasites (trematodes, cestodes, nematodes) that affect domestic and wild animals, and even humans. For example, snails have been identified as responsible for the transmission of the pathogen *Phytophthora citricola* in avocado (El-Hamalawi and Menge, 1996), and slugs as responsible for the spread of *Sclerotinia trifoliorum*, which causes infections of white clover (Barker, 2002). It has also been shown that slugs and snails, whose faeces frequently contaminate freshly consumed vegetables, act as vectors of *Escherichia coli*, as bacteria can survive on their faeces for up to 3 weeks (Sproston *et al.*, 2006).

A number of species of terrestrial gastropods are considered as agricultural pests because they cause serious damage to plants cultivated by man. These animals affect a wide variety of species and productive sectors, from large intensive crops to public and private gardens, including horticulture, floriculture, forestry, fruit growing, grassland and commercial crops of ornamental, aromatic and medicinal plants (Speiser, 2002). According to Barker (2002), land gastropods are currently one of the most difficult problems for an agriculture that claims to be sustainable. Terrestrial gastropod pest control in organic farming is especially difficult, making these animals the most harmful to organic crops according to professional associations in UK and Switzerland (Peackock and Norton, 1990; Kesper and Imhof, 1998). More recently, Douglas and Tooker (2012) also pointed out that slugs are challenging to control because of the limited number of management tactics that are available. Most of the research carried out on the importance of terrestrial gastropods as agricultural pests and their control has been carried out in Europe during the last third of the previous century, but publications on this topic have become increasingly frequent in recent years from other geographic areas (Prokop, 2005; Naranjo-García *et al*., 2007; Nash *et al*. 2007; Salvio *et al.*, 2008; Clemente *et al.*, 2008; Hoffmann *et al.*, 2008; Micic *et al.*, 2008; McDonnell *et al.*, 2009; Ross *et al.*, 2010; Eskelson *et al.*, 2011; Douglas and Tooker, 2012; Douglas *et al.*, 2015), which demonstrates an increased concern about the negative impact of these animals worldwide. On the other hand, some studies suggest that the abundance, range and intensity of damage caused by some species of terrestrial gastropods will increase in coming years due to the effects of climate change (Willis *et al.*, 2006; Capinha *et al.*, 2014).

In economic terms, damage caused by terrestrial gastropods in agriculture is considered to be smaller than that caused by other pest organisms, such as insects, mites, nematodes or fungi, but in certain geographic areas and crops gastropods are responsible for very large crop losses (Barker, 2002). While some land snails can reach pest status even in relatively arid regions, slugs are especially problematic in temperate and rainy climates. One of the main characteristics of the damage caused to crops by these animals is that their magnitude varies widely at regional level and from year to year (Port and Port, 1986). Overall, the majority of specialists agree that the damage caused by gastropods has significantly increased in recent decades, due to factors such as the simplification of cultivation techniques (prohibition of burning straw, reduction of tillage, direct drilling, increased use of set-aside), reduction of insect populations due to abusive use of insecticides, the use of new crop varieties more susceptible to attack by gastropods, or the accidental or intentional introduction of alien species of gastropods in many regions of the world (Hommay, 1995, 2002; Godan, 1999; Speiser, 2002; Barker, 2002).

Direct damage caused by terrestrial gastropods in agriculture is related, depending on the crop in question, to one or more of the following aspects:

- reduction of crop volume due to the consumption of seeds, seedlings, roots or aerial parts of adult plants.
- loss of quality of the harvested products by the diminution of its size or vigour.
- loss of quality due to "cosmetic damage" (quantitative small damages that affect the appeal of the harvested products).

In short, what is affected is the economic value of the crop. The so-called cosmetic damages acquire special relevance in the sectors of horticulture and ornamental plants, where the external appearance of the final product is of great importance to the consumer, so that any small damage or malformation, or the presence in the product of the pest animals, their faeces, mucus or eggs, can lead to the loss of much of its economic value (Port and Ester, 2002).

In large monocotyledonous (cereals, forage crops) and dicotyledonous (rape, sunflower, soybeans, beets) crops, the most significant damage caused by gastropods is due to the killing of seeds after sowing and the destruction of seedlings, which leads to a significant reduction in the number of plants harvested (Port and Port, 1986). In France, 12.5% of the maize cultivated area, and 53% of sunflower cultivated area, are systematically treated with molluscicidal products (Hommay, 2002). In Great Britain, on the other hand, terrestrial gastropods have been estimated to cause on average a reduction of 2% in the annual wheat production (Glen and Moens, 2002), and that the rape crops treated with molluscicides have increased from 6% to 58% in the period 1977 to 1996 (Moens and Glen, 2002); furthermore, consumption of molluscicides in the UK increased by a factor of 67 between the early 1970s and mid-1990s (Garthwaite and Thomas, 1996). Crop losses caused by slugs have been estimated in approximately £8 million each year in Britain (Port *et al.*, 2003).

Horticultural crops affected by terrestrial gastropods are mainly brassicas (all kind of cabbages, Brussels sprouts, cauliflower, broccoli, etc.), but also others such as lettuce, carrots, asparagus, peppers, celery, strawberries, etc., frequently suffer the attack of these molluscs (Port and Ester, 2002). In horticultural land there is a low degree of soil disturbance and a greater diversity of habitats and plant species, factors which favour populations of gastropods. In addition, the tolerance of horticultural crops to damage caused by gastropods is generally very low, due to the great importance of the cosmetic component (Port and Ester, 2002). A clear example is represented by Brussels sprouts: it has been estimated that a single slug (*D. reticulatum*) damages between 4 and 8 sprouts per night, and that even with the application of molluscicides, between 60% and 80% of the harvested sprouts usually present some damage (Godan, 1973); however, in order to be considered of superior quality, a consignment of Brussels sprouts should not show more than 5% of damaged sprouts, even if the damage is merely superficial. In the case of cabbages and lettuces, slugs not only cause damage to the outermost leaves, which have to be removed before being marketed, but also take shelter inside and damage the innermost leaves; it has been observed that even in the conservation chambers, at temperatures 1 to 5 °C, the slugs sheltered inside these vegetables continue to feed on them (Port and Ester, 2002).

In grassland, the impact caused by terrestrial gastropods is less known, although grassland usually harbours abundant and diverse communities (Barker, 2002). It has been pointed out that the selective predation of the gastropods on the seeds and seedlings of grassland vegetation interferes with the natural process of regeneration of prairies, with its most important effect being the preferential consumption of legumes like *Trifolium* spp., *Lotus* spp., or *Medicago* spp., whose role in the productivity of the pasture is paramount: on the one hand, they contribute directly to the nutrition of livestock because their protein content and digestibility are superior to those of the grasses and, on the other hand, legumes make an extremely important indirect contribution to the primary productivity of the whole system because of its role in nitrogen fixation, which is the most limiting element of grassland productivity (Barker, 2002). Barker *et al.* (1985), and Barker and Addison (1992) showed that populations of the slug *D. reticulatum* with densities of 20 to 80 individuals m^{-2} produce a significant reduction of the leaf surface of *Trifolium repens* in New Zealand grassland, and observed that regular treatments with molluscicides produce an increase in *T. repens* coverage of up to 40%. Baker (1989) observed that the presence of populations of the snail *Theba pisana* can reduce by 23% the primary production in South Australia prairies, and by 75% the production of *Trifolium* spp; in this region, losses caused by gastropods in the production of permanent grasslands have been estimated as the equivalent of the vegetation consumed per hectare by 0.5 to 2.5 sheep.

THE PEST SLUG *Deroceras reticulatum*

Taxonomy and distribution

The Mollusca are in appearance, anatomy, ecology, and physiology a highly diverse group, for which the phylogenetic pathways and higher classification have been controversial since the very beginning of comparative investigation. Within the Mollusca, most systematic problems arise among the ecologically most diverse Gastropoda, where most members are marine, but several lineages have colonised freshwater and/or terrestrial environments, and as a consequence the taxonomy and nomenclature of Gastropoda have always been particularly problematic and unstable (Barker, 2001).

As regards taxonomy, we have followed the recent *Classification and Nomenclator of Gastropod Families* (Bouchet and Rocroi, 2005), a working classification attempting to reconcile recent advances in the phylogeny of the Gastropoda, which use unranked clades above the superfamily level, and more traditional approaches which use hierarchical ranking and follow the International Code of Zoological Nomenclature for the superfamily and lower levels. For example, the Pulmonata gastropods, which are the molluscs that have radiated most extensively in terrestrial habitats, have been historically accepted as monophyletic, and many morphological as well as molecular analyses supported the monophyly of the Pulmonata; however, some phylogenetic analyses of 18S and 28S rDNA sequences did not confirm its monophyly, and the analyses of Grande *et al*. (2004) based on several mitochondrial gene sequences indicated with strong support that the Pulmonata are polyphyletic; thus, the traditional taxon Pulmonata Cuvier in Blainville, 1814 is treated as “informal group Pulmonata” by Bouchet and Rocroi (2005). The systematic framework of Bouchet and Rocroi (2005) is currently followed by many authors and it is expected that it will be the standard in the scientific community for the next decades (Gargominy *et al*., 2011).

According to the system proposed by Bouchet and Rocroi (2005), the systematic position of *D. reticulatum* is as follows:

Phylum Mollusca Cuvier, 1795
Classis Gastropoda Cuvier, 1795
Subclassis Orthogastropoda Ponder y Lindberg, 1995
Clade Heterobranchia
Informal group Pulmonata
Clade Eupulmonata
Clade Stylommatophora
Informal group Sigmurethra
Limacoid clade
Superfamilia Limacoidea Lamarck, 1801
Familia Agriolimacidae H. Wagner, 1935
Subfamilia Agriolimacinae H. Wagner, 1935
Genus *Deroceras* Rafinesque, 1820
Subgenus *Deroceras* Rafinesque, 1820
Deroceras (Deroceras) reticulatum (O.F. Müller, 1774)

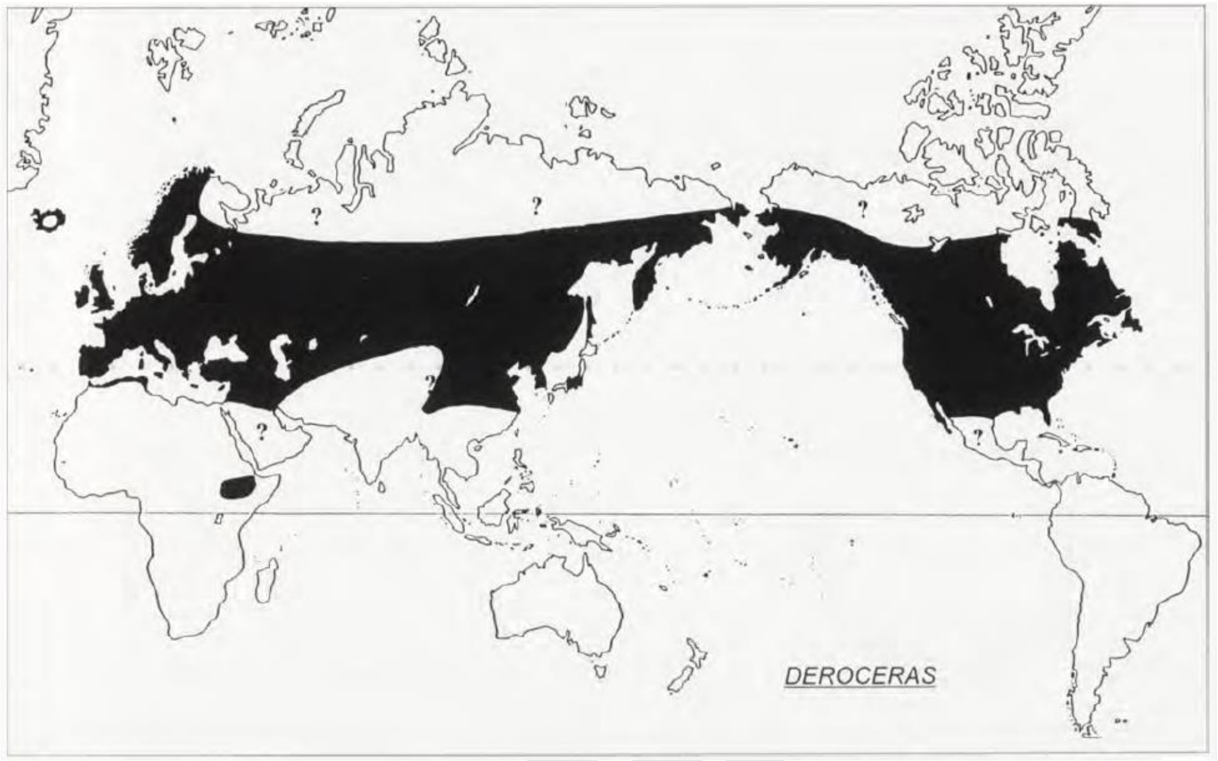

Figure 1. Distribution range of indigenous *Deroceras* species (after Witkor, 2000).

Members of the Stylommatophora are strictly terrestrial and constitute the dominant group of Pulmonate molluscs on land, thanks to a suite of morphological, physiological and behavioural adaptations that enable a high degree of regulation of body hydration, such as the contractile pneumostoma, uricotelia, a remarkable physiological tolerance to variation of body water content, and a set of behaviours that allow them to survive in unstable environments while maintaining their ability to function effectively (Barker, 2001; Cook, 2001). Stylommatophorans are primarily snails, but the slug form has evolved many times in gastropods living in marine and terrestrial habitats (a process termed `limacisation' by Solem 1974), and many taxa of widely divergent origins have, by parallel evolution, assumed a remarkable similarity in their sluggish aspect (Barker, 2001). Within the Stylommatophora there are families composed only of snail forms, families composed of both snails and slugs, and families composed only of slugs. The family Agriolimacidae comprises only slugs and is the most speciose and widest distributed slug family (Wiktor, 2000). Agriolimacids occur indigenously only in the northern hemisphere; their range covers nearly whole Eurasia, from the Polar Circle up to the Himalayas and Central America, while in Africa they are delimited by the Sahara, even when a few endemic species of *Deroceras* inhabit Ethiopia. Within this wide range, the number of agriolimacid species is always low except for the Mediterranean area, suggesting that the historical centre of dispersal of the family was situated somewhere in the south-western Paleartic. The genus *Deroceras*, represented by nearly 120 species, is the only one found in the whole vast area covered by the family (Figure 1), with the highest number of *Deroceras* species inhabiting the Mediterranean region, especially the Balkans. However, several *Deroceras* species have been dispersed almost worldwide through human activities, and this is particularly true in the case of *D. reticulatum* (Barker, 1999, 2001; Wiktor, 2000). Currently, *D. reticulatum* is found in most temperate and subtropical regions, including Europe, Asia, Australia, New Zealand, Tasmania, Canada, the United States, South America, South Africa and many islands of the Pacific and Atlantic Oceans (Forsyth, 2004).

Biology

Deroceras reticulatum (Figure 2) is a medium-sized slug up to 45-50 mm in length when fully extended, with a short, ill-defined keel. Mantle shield about 0.3 of body length. Body wall thick, usually cream or pale brown, with blackish or brown spots distributed mainly in the skin grooves. Mantle similarly cream or pale brown, flecked or spotted black or brown. Pigmentation varying within populations from wholly dark to pale specimens. Border of pneumostome pale. Sole usually creamy, but brown in intensely coloured specimens. Mucus clear in undisturbed animals, but milky on irritation. Genital orifice immediately posterior to right ocular peduncle (Castillejo, 1997; Barker, 1999; Wiktor, 2000).

The species shows a wide ecological tolerance, often found in anthropogenic, disturbed areas such as agricultural land and ruderal habitats (Runham and Hunter, 1970). *D. reticulatum* is a non-aggressive species, both at intraspecific and interspecific levels, which offers little competition to other slug species in stable environments (Rollo and Wellington, 1979; McCraken and Selander, 1980). However, its wide-ranging tolerance to factors such as temperature, soil moisture or food availability enables it to out-compete other slug species in unstable environments (Willis *et al.*, 2008). Furthermore, *D. reticulatum* is able to reproduce throughout the year in temperate climates (Carrick, 1938; Barnes and Weil, 1945; South, 1989; Córdoba *et al.*, 2011). As a consequence of its traits, *D. reticulatum* is a successful opportunistic and invasive species (Port and Port, 1986; South, 1992), and the most serious slug pest worldwide (Kerney and Cameron, 1979; Speiser, 2002; Grimm and Schaumberger, 2002). Everywhere it occurs, *D. reticulatum* is generally the most abundant slug in modified habitats (Barker, 1999).

Figure 2. *Deroceras reticulatum*.

Like most slugs, *D. reticulatum* is preferentially a nocturnal animal, beginning its activity around sunset, when the rate of the fall of temperature is slower (Dainton, 1989). The activity and abundance of *D. reticulatum* is strongly affected by temperature and other weather factors such as air humidity, soil moisture, rainfall or wind speed (Cook, 2001). However, the activity of *D. reticulatum* is not restricted by cold conditions to the same extent as for other slug species, and it is known to feed normally at temperatures as low as 0 ˚C (Crawford-Sidebotham, 1972). *D. reticulatum* is a hermaphroditic, semelparous, outcrossing species, and mature individuals often evidence their sexuality by a prominence or dilation of the genital orifice; animals in this state are not infrequently observed, in the field and in laboratory cages, to approach conspecifics in an attempt to arouse a mating response (Barker, 1999). They lay their eggs in the soil surface or under leaves, in batches with an average of 22 eggs per batch, and can lay up to 500 eggs over its life (Carrick, 1938; Port and Port, 1986). Temperature and soil moisture influence strongly the oviposition behaviour, and the range of temperature and moisture conditions over which *D. reticulatum* will oviposit has been pointed out by Carrick (1942) as being between 3 and 20 ˚C, and between 25 and 100% saturation of soil with moisture, with 10–20 ˚C and 64% soil moisture being optimal. More recently, Willis *et al.* (2008) demonstrated that *D. reticulatum* is able to adjust its egg laying to the surrounding temperature and moisture conditions, and thus to alter their investment in reproduction to maximise fitness, with most eggs being laid at a combination of 53% soil moisture and 18 ˚C. The incubation period strongly depends on temperature, and ranges from 175 days at 4.4 ˚C to 15 days at 20 ˚C (Carrick, 1942). The young slugs hatch fully formed, breaking their way out of the egg by rasping at its envelope with the radula, and neonate slugs have been observed to eat the remains of their own eggs and other neighbouring intact eggs (Carrick, 1938). The maximum life span of *D. reticulatum* is 12 month or so (South, 1992), and the generation time can be as short as 6 month in the field (South, 1992; Barrada *et al.*, 2004a). *D. reticulatum* feeds on a wide range of plants and is usually regarded as a generalist herbivore or as omnivore, although it is capable of exhibiting distinct preferences for different food items and plant species (Wiktor, 2000; Barrada *et al.*, 2004b).

Its reproductive phenology vary geographically and from year to year in the same location, but reproductive activity and abundance are generally higher in autumn and spring, in reaction to favourable weather conditions (Barker, 1999). Even so, a number of studies have shown that *D. reticulatum* will reproduce whenever environmental conditions permit (Carrick, 1938; Barnes and Weil, 1945; South, 1989; Córdoba et al., 2011), as is expected for opportunist species (Willis *et al.*, 2008). The population structure of *D. reticulatum* is such that there is often more than one overlapping generation per year, resulting in individuals at the oocyte stage being present most of the time (Hunter and Symonds, 1971; Barrada *et al.*, 2004a), which further allows reproduction whenever conditions are favourable. Individuals are therefore present throughout the year and are potentially always capable of causing damage to crops.

CONTROL OF PEST SLUGS

Chemical control

Except otherwise stated, this epigraph is based on South (1992), Bailey (2002), Henderson and Triebskorn (2002) and Speiser (2002).

There are documents recommending using salt to kill slugs and snails from as early as 1349. Different mixtures with salt, sand, caustic soda, lime, sawdust, soap and other products were recommended in a pamphlet of the British Ministry of Agriculture from 1905. More recently, a number of authors suggested inorganic salts such as copper sulphate, aluminium sulphate, iron sulphate, calcium arsenate, etc., applied as powder or spray; another traditional way of control is the use of metallic barriers made of cooper or zinc. All these forms of control are based on the irritating effect of the substances when in contact with the tegument of gastropods, causing in the slugs and snails a large production of mucus and dehydration, so that they avoid contact with the chemicals. However, after an initial reaction contact with the substances, most of the animals recover soon, and under conditions of high humidity all these methods lost most of their effectiveness.

At present, the most common method of slug control is the use of chemical molluscicides. This are usually pellets or baits containing between 2% and 8% of either metaldehyde or methiocarb as active ingredient. In most countries, metaldehyde is the commonest molluscicide used. In Britain, metaldehyde baits are used in 55% of the crops treated against slug pests, compared to 40% treated with methiocarb. Metaldehyde is a cyclic tetramer of acetaldehyde. Its molluscicidal activity was discovered by chance, because it was originally sold as solid fuel (meta-tablets) and French farmers accidentally noticed dead slugs where meta-tablets had been left on the ground. It was introduced as a molluscicide in 1936 and was first used in slug baits in the early 1940s. Metaldehyde has molluscicidal activity both by ingestion and dermal contact. Its initial effect is just irritating, causing the slug to produce abundant mucus secretion and dehydration, but it has also a neurotoxic effect which ends in slug paralysis. Metaldehyde pellets lost effectiveness under conditions of high humidity and/or low temperature, and tends to depolymerize rapidly under acid pH conditions. Thus, it shows less effectiveness in areas of high soil humidity and acid soils, such as most Galicia.

The other widely used molluscicide is methiocarb. Like other compounds belonging to the family of carbamates, methiocarb causes metabolic alterations in insects, molluscs and warm-blooded animals, and inhibits acetylcholinesterase in the nervous system interfering with nerve impulse transmission. Methiocarb poisoned slugs do not show mucous secretion or dehydration, but a reduction in feeding activity, a loss of muscle tone and swelling up with fluid becoming immobile until they die. Methiocarbs is particularly effective in wet weather and low temperatures, when the slugs show higher activity. Its main drawback is that is highly toxic to other organisms such as earthworms, insects, birds, and mammals.

Research showed that molluscicidal baits with metaldehyde have a negative impact on populations of some carabid beetles, while negative effects on both carabids and staphylinids populations have been shown after treatments with molluscicidal baits with methiocarb. Furthermore, cases of poisoning of domestic and wild animals due to the consumption of molluscicidal baits with either metaldehyde or methiocarb are common, even when molluscicidal baits incorporate pigments (usually blue or green) and other substances like bitrex (an extremely bitter-tasting substance) to reduce the risk of ingestion by mammals and birds. Baits with methiocarbamate have been shown to pose a serious threat to populations of rodents such us the field mouse *Apodemus sylvaticus*. Molluscicidal baits with carbamates were banned in some states of North America by the end of the 1980s, due to recorded high frequencies of bird poisoning. Also, high concentrations of acetaldehyde (resulting from the depolymerisation of metaldehyde in the digestive tract have been reported in hedgehogs (*Erinaceus europaeus*) in the field, as well as poisoning symptoms and cases of death in hedgehogs fed with slugs that had previously ingested baits with methiocarb.

In the las two decades, a new chemical molluscicide has been marketed in Europe and North America, containing iron phosphate as active ingredient. The mode of action of iron phosphate is not fully understood. In slugs and snails, iron phosphate acts as a stomach poison, damaging their digestive tissues, so that they stop eating and slowly die. Tests conducted to verify its effectiveness (Iglesias and Speiser, 2001; Speiser and Kistler, 2002) showed that it is comparable to the classical molluscicides, metaldehyde and methiocarb. However, unlike those chemically-synthesized compounds, iron phosphate naturally appears as a part of several minerals, especially strengite and metastrengite, and has a low toxicity

(EPA, 1998), to the point that it is authorized for use in organic farming in some countries. Notwithstanding, recent research have shown that molluscicide pellets containing iron phosphate have adverse effects on earthworm survival, activity and growth (Langan and Shaw, 2006; Edwards *et al.*, 2009). The adverse effects seem to be caused by synthetic chelating agents added to the pellets (such as EDTA, ethylene diamine tetracetic acid, or EDDS, ethylene diamine succinic acid), which solubilize the iron contained in the iron phosphate and make it more available and toxic (Edwards *et al.*, 2009). The chelating agents, in combination with iron phosphate, present significant hazards not only to earthworms and other soil inhabiting invertebrates, but to domestic animals and human child (Edwards *et al.*, 2009).

Biological control and natural enemies

Except otherwise stated, this epigraph is based on Barker (2004).

Slugs and snails are preyed upon by a variety of vertebrate and invertebrate natural enemies. Vertebrate predators include birds, amphibians, reptiles and mammals. Among European birds, wild species of Turdidae (*Turdus* spp.), Corvidae (*Corvus* spp., *Pica pica, Garrulus glandarius*), Sturnidae (*Sturnus vulgaris*) are regular consumers of snails and slugs, although the quantitative contribution of molluscs to their diet is low; poultry, including chickens, ducks and geese eat large numbers of slugs if available, and the release of poultry in gardens, orchards and crops as a means of protection against gastropod damage is a popular practice, even when poultry also can cause significant damage to plants. With respect to amphibians and reptiles, it is known that some toads (*Bufo bufo*), salamanders (*Salamandra salamandra*), and the lizard *Anguis fragilis* eat slugs regularly. Hedgehogs (*Erinaceus europaeus*), moles (*Talpa europaea*), shrews (*Sorex* spp., *Crocidura* spp.), rodents (*Rattus* spp., *Apodemus sylvaticus*), badgers (*Meles meles*), wild pigs (*Sus scrofa*) and foxes (*Vulpes vulpes*) stand out among mammals as consumers of terrestrial gastropods. Although vertebrate predators undoubtedly make a contribution to the control of terrestrial gastropods, it is unlikely that the population densities of these predators are large enough to significantly influence slug and snail populations in the field.

Among invertebrates, the main natural enemies of terrestrial gastropods are arthropods, especially ground beetles (Carabidae), rove beetles (Staphylinidae), fireflies (Lampyridae), burying beetles (Silphidae), false firefly beetles (Drilidae), marsh flies (Sciomyzidae), blowflies (Calliphoridae), flesh flies (Sarcophagidae), harvestmen (Opiliones), and centipedes (Chilopoda). A number of Nematode species, mainly within the Rhabditidae, Angiostomatidae, Alloionematidae and Cosmocercidae, and a few species of mites (Acari), flatworms (Platyhelminthes), and carnivorous terrestrial gastropods, complete the list of invertebrates that feed on terrestrial molluscs, either as parasites or as predators. Carabid beetles and nematodes are the most important natural enemies of terrestrial gastropods and the most promising biological control agents. Some species within Fungi, Ciliophora (Protista) and Bacteria are known to be pathogens of terrestrial gastropods, and may be important natural enemies of pest slugs and snails, but have been poorly studied.

Carabid beetles are widely recognized as beneficial organisms in agroecosystems and are increasingly regarded as crucial for regulating many pest populations, as many synthetic pesticides are phased out (Berthe *et al.*, 2015). There are predatory carabid species that are considered gastropod specialist, such as *Cychrus caraboides* and *Carabus violaceus*, although studies have demonstrated that even these species feed on other prey items. Specialist, or at least stenophagous natural enemies are usually employed in classical biological control introductions, while conservation biological control rely mainly on generalist species, and it is known that many generalist carabids kill and eat slugs and snails in the field. Here, we follow Symondson *et al.* (2002) in considering synonymous the terms generalist, polyphagous, unspecialised, and omnivorous. Many unspecialized ground beetles which are common and abundant in agricultural land eat almost any prey it can subdue, including a wide range of arthropods, molluscs and annelid worms, and also feed on plants and fungi. The great influence of carabid beetles on slugs is reflected in some slug behaviours. For example, slugs are capable of detecting the presence of ground beetle species that regularly consume slugs, presumably via olfactory cues, and alter their behaviour by becoming less active (Armsworth *et al*., 2005), and can even discriminate amongst different predators and adjust their behavioural response according to the relevance of the threat (Bursztyka *et al.*, 2013). When attacked, slugs produce copious quantities of defensive mucus, which can gum up the mouthparts and even the legs of arthropod predators. Other defensive behaviours include tail-wagging, escaping by descending on a mucus thread, and occasional autotomy of the tail.

The only biological control agent ever marketed for the control of terrestrial gastropods is the bacterial-feeding, rhabditid nematode, *Phasmarhabditis hermaphrodita,* first launched to the market in the UK in 1994. Similar to many entomopathogenic nematodes, *P. hermaphrodita* enters its host as an infective juvenile (dauer larvae, equivalent to the L3 larval stage) associated with bacteria that are thought to be largely responsible for its pathogenicity. Infective dauer larvae actively search the soil for hosts such as *D. reticulatum*, following chemical cues, and when a host is found, the larvae enter the shell sac of the slug through a dorsal integumental pouch located in the posterior region of the slug mantle. Then the larvae develop into hermaphroditic self-fertilising adults and reproduce. Parasitized slugs develop a swelling of the mantle area and often shed their internal shell through a hole in the swollen mantle. Infested *D. reticulatum* soon stop feeding, and die between 4 and 21 days after becoming infected. The rapid inhibition of slug feeding enhances its effectiveness in preventing crop damage. After the death of the slug, the nematodes spread and reproduce over the cadaver until this food source is depleted. Then the nematodes fail to complete their life-cycle and form new non-feeding dauer larvae that seek through the soil for a new host or other food source, since *P. hermaphrodita* is capable of living on other decaying hosts, slug faeces or leaf litter. In its European home range, where this nematode is common and widespread, it is believed that wild *P. hermaphrodita* realize a natural control of the populations of susceptible slug species (Rae *et al.*, 2007).

Nematodes are sold in sealed packs containing either 6 or 12 million dauer larvae interspersed with calcium montmorillonite clay. The packs must be stored in the refrigerator and have expiry date. To be used, the content of the pack is mixed with water and sprayed or watered into the ground, at a rate of 300000 larvae per square meter (allowing the treatment of either 20 or 40 m^2). Numerous field trials conducted in a wide variety of crops in different countries have shown that *P. hermaphrodita* is able to reduce the damage caused by slugs in agriculture and horticulture. Its effectiveness against *D. reticulatum* is beyond doubt, but its effect against other, larger slug species, may be lower. Although the effectiveness of *P. hermaphrodita* is highly dependent on soil temperature and moisture, which affect nematode survival, it has the advantage that conditions that favour nematode survival also promote slug activity, increasing the chance that slugs and nematodes get in contact. Unfortunately, the short shelf life and high economic cost of *P. hermaphrodita* restricts its use to high-value crops such as ornamental plants and some vegetables.

Other control methods

Except otherwise stated, this epigraph is based on South (1992) and Speiser et al. *(2001).*

There are a number of alternative, manual methods proposed to control slug populations and/or reduce slug damage. They include hand-picking of slugs at night, the use of different kinds of pit-fall traps (most frequently baited with beer) where the slugs fall and die, or artificial shelters where slugs can be found during the day to be collected and killed. Of course, these methods are only feasible for small areas such as gardens.

For large crops, cultural practices that reduce slug activity are recommended, but they are sometimes labour-intensive, and may influence the performance of crops or affect the environment, which often limits their application. In general, rough seed beds and continuous, dense vegetation cover favour slugs, and should therefore be avoided. Good soil cultivation can reduce slug populations drastically. Machinery, timing and intensity of cultivation have to be adjusted to the soil type and to the needs for soil conservation. The objective is to obtain a fine seed bed, which reduces movement of slugs between their shelter in the soil and the crops. Soil cultivation at low temperatures exposes slugs or slug eggs to freezing. Sowing seeds deeper than usual protects seed against slug damage. Sowing or planting susceptible crops at a distance of at least 5 m from field borders (fallows, meadows or hedges) reduces damage caused by immigrating large slugs. Optimal fertilization and irrigation methods, the use of vigorous cultivars, and changes in sowing or planting dates may reduce the duration of the phase when crops are most susceptible to slugs. It is well known that different cultivars of both horticultural and agricultural crop species show different degrees of susceptibility to slug damage, but slug resistant cultivars do not exist yet.

CLIMATE CHANGE AND PREDICTED EFFECTS ON AGRICULTURAL PESTS

In recent decades, changes in climate have caused significant impacts on different ecosystems on all continents (IPCC, 2014). Overall, climate models for Europe predict significant warming, changes in the rainfall pattern and an increase in the frequency of extreme weather events, which are likely to cause significant impacts in agro-ecosystems (Olesen *et al.*, 2011). Pests and pathogens are currently responsible for crop losses of up to 40% worldwide (Oerke, 2006), and up to 25–30% in Europe and the USA, and these values are predicted to rise as a result of global warming (Maxmen, 2013). As a consequence, there is growing concern that global food security is threatened by the emergence and/or spread of crop pests and pathogens (Bebber *et al.*, 2013). The links between climate change and food security are receiving increasing attention, as the role of pests in limiting the production of food, fiber, and feed is universally acknowledged (Ziska and McConnell, 2016).

The expected warming in Europe is greatest over the eastern countries during winter, and over western and southern Europe in spring-summer; thus, projected increase in spring-summer temperatures could exceed 6 ˚C in parts of France and the Iberian Peninsula by the end of the 21st century under the A2 emission scenario, which describes a heterogeneous world with high population growth, slow economic development and slow technological change (Olesen *et al.*, 2011).

Temperature is considered the most dominant environmental factor affecting the biology of ectotherms (Taylor, 1981; Brown *et al.*, 2004), including important reproductive traits such as developmental rate, survival or fecundity, and therefore it is expected that global warming will have a deep impact on their population dynamics (Zidon *et al.*, 2016). Climate change will lead to invasions and spread of pests and diseases adapted or able to survive under warmer climatic conditions, and evidences show that dispersal rate of pests and diseases are most often so high that their geographical extent is determined by the range of climatic suitability (Baker *et al.*, 2000). Moreover, studies suggest that species that produce large numbers of offspring that grow and reproduce quickly but are small-sized and have a short life-span (i.e. r-strategists, such as most pest species) are prone to become successfully established outside their native regions and climate conditions (Sakai *et al.*, 2001). Thus, during the last decade multiple reports have shown that climate change is already altering the distribution (Kocmánková *et al.*, 2011; Capinha *et al.*, 2014; Gilioli *et al.*, 2014), behaviour (Dalin, 2011; Hoffmann and Sgrò, 2011) and/or population dynamics (Willis *et al.*, 2006; Altermatt, 2010; Zidon *et al.*, 2016) of many agricultural pests around the globe.

A classic approach to study the sensitivity of a species to temperature changes utilize thermal performance curves (Huey and Stevenson 1979), which are asymmetric functions describing the relationship between temperature and the intrinsic rate of population growth or fitness of the species (Kingsolver, 2009). These curves combine the effects of fitness indicators, such as fecundity, growth, or survivorship, and allow the identification of the species lower and upper temperature limits, the temperature of maximum performance, the species thermal breadth (Gilchrist, 1995) and its warming tolerance (Kingsolver *et al.*, 2013). Another approach to predict the impact of climate changes on the species general performance and fitness is the utilization of temperature-dependent population dynamic models for simulating the species dynamics in the field under different temperature scenarios, which is generally achieved by running established degree-day models with projections from climate models (Gilioli *et al.*, 2013). However degree-day models assume a linear response of population parameters to temperature and might be limited in their ability to accurately predict population outbreaks and risks of pest populations dynamics, and more complex models of population dynamics comprising several demographic variables effecting population performance, in combination with stochastic models of climate change under different scenarios have been developed (Zidon *et al.*, 2016).

Climate change will not only affect the pest species of concern, but will also affect the biology of their natural enemies, the quality and/or quantity of available food, and many other factors. In the agricultural context, climate change is expected to affect also the varieties and species of crops grown, as well as the timing of agricultural practices (Olesen *et al.*, 2011). All together throw a high degree of uncertainty on the forecasting of the responses of any particular species to future climatic conditions (Wilson *et al.*, 2015). It is becoming more and more evident that forecasting should take into account not only the pest species, but also their natural enemies and ecological relationships (Lepetz *et al.*, 2009). However, for modelling projections to be accurate they need to be parameterised and validated by experimental evidence (Capinha *et al.*, 2014; Berthe *et al.*, 2015). Experimental manipulations of temperature and precipitation have already provided insights into the responses of terrestrial ecosystems to climate change, with warming generally increasing total net primary productivity (NPP) and photosynthesis (Wu *et al.*, 2011). However, such studies have mostly focused on the responses of plants to simulated climate warming, and few have considered the response of animal communities, particularly in agro-ecosystems. Among the first to deal with this topic, Sternberg (2000) found that artificial winter warming in grassland caused an increase in *D. reticulatum* abundance due to increased winter survival, while increased summer rainfall caused a decrease in slug numbers associated with changes in the surrounding vegetation which reduced the preferred food of the slugs. More recently, Dong *et al.* (2013) demonstrated that experimental warming of wheat fields can lead to an increase in pest aphid abundance. Buchholz *et al.* (2013) showed increases in ground beetle, spider and grasshopper activity-densities, but lower diversity, under experimental grassland drought and warm conditions. Similarly, Berthe *et al.* (2015) showed that artificial warming by 2 ˚C in a spring-sown wheat crop caused a reduction in overall diversity of coleopterans but and increase in activity-densities of the four most common species. All these studies have provided valuable information into the responses of different components of terrestrial ecosystems to predicted climate change, as a fundamental previous step for modelling the functional response of agro-ecosystems to climate change at the farm-level or wider scales.


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AIM AND OBJECTIVES


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AIM AND OBJECTIVES

The aim of the PhD work presented here is to explore potential changes in the feeding behaviour of the most serious slug pest worldwide, the grey field slug *Deroceras reticulatum*, as well as in the behaviours of some of their most important natural enemies, predatory carabid beetles and parasitic nematodes, under climatic conditions predicted for Galicia by the last third of the century. To achieve this general aim, different experiments under laboratory controlled conditions and under semi-natural conditions have been performed. In particular, the focus has been placed on shedding light on the following specific objectives:

1) Whether, and to what extent, predicted climatic conditions affect the feeding activity of *Deroceras reticulatum* populations and their potential to damage crop seedlings, which are often the most vulnerable stage of crops to slug damage. Chapter 1.

2) Whether, and to what extent, the carabid beetle *Harpalus rufipes*, one of the most common and abundant ground beetle in Europe, can destroy eggs and different sized slugs of the slug *Deroceras reticulatum*. Chapter 2.

3) Whether, and to what extent, temperature and predicted climatic conditions affect the predatory activity of carabid beetles on the eggs of *Deroceras reticulatum*. Chapter 3.

4) Whether, and to what extent, predicted climatic conditions affect the performance of the unique biological control agent currently marketed against terrestrial gastropods, the nematode *Phasmarhabditis hermaphrodita*. Chapter 4.


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CHAPTER 1

El-Danasoury H., Iglesias-Piñeiro J., Córdoba M. (2016) The effect of climate manipulations on the herbivory of the pest slug *Deroceras reticulatum* (Müller, 1774) (Pulmonata: Agriolimacidae). *International Journal of Biometeorology*, **60**, 1501-1507.
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The effect of climate manipulations on the herbivory of the pest slug Deroceras reticulatum (Müller, 1774) (Pulmonata: Agriolimacidae)

H. El-Danasoury, J. Iglesias-Piñeiro, M. Córdoba

Abstract

The pestiferous status of the terrestrial slug *Deroceras reticulatum* and the strong dependence of its biology and ecology on climatic factors have driven research on the potential responses of the slug to predicted scenarios of climate change. Here, we report two short-term experiments performed outdoors, under seminatural conditions, to assess the behavioural response of *D. reticulatum* to different climate manipulations in terms of herbivory, by measuring over 7 days the damage inflicted by slug populations to lettuce seedlings. The climate manipulations tested emulate predicted climatic conditions for northwest Spain, specifically winter warming and increased summer rainfall, in contrast respectively with normal winter conditions and summer without rain conditions. In a winter experiment, we compared a normal winter treatment with a winter warming treatment; with respect to the normal winter treatment, the winter warming treatment was characterised by higher temperature, lower relative humidity and the absence of rainfall. In a summer experiment, we compared a summer drought treatment with an increased summer rainfall treatment; with respect to the summer drought treatment, the increased summer rainfall treatment was characterised by the presence of rainfall, while the conditions of temperature and relative humidity were similar in both treatments. Neither winter warming nor increased summer rainfall did lead to a significant increase on the number of seedlings damaged by the slugs. However, with both treatments, we found a moderate increase on the amount of damage suffered by the seedlings. The results are discussed in the context of the potential responses of *D. reticulatum* to future climatic conditions.

Keywords: *Deroceras reticulatum*, herbivory, crop damage, climate manipulations, behavioural change.

http://link.springer.com/article/10.1007/s00484-016-1140-0

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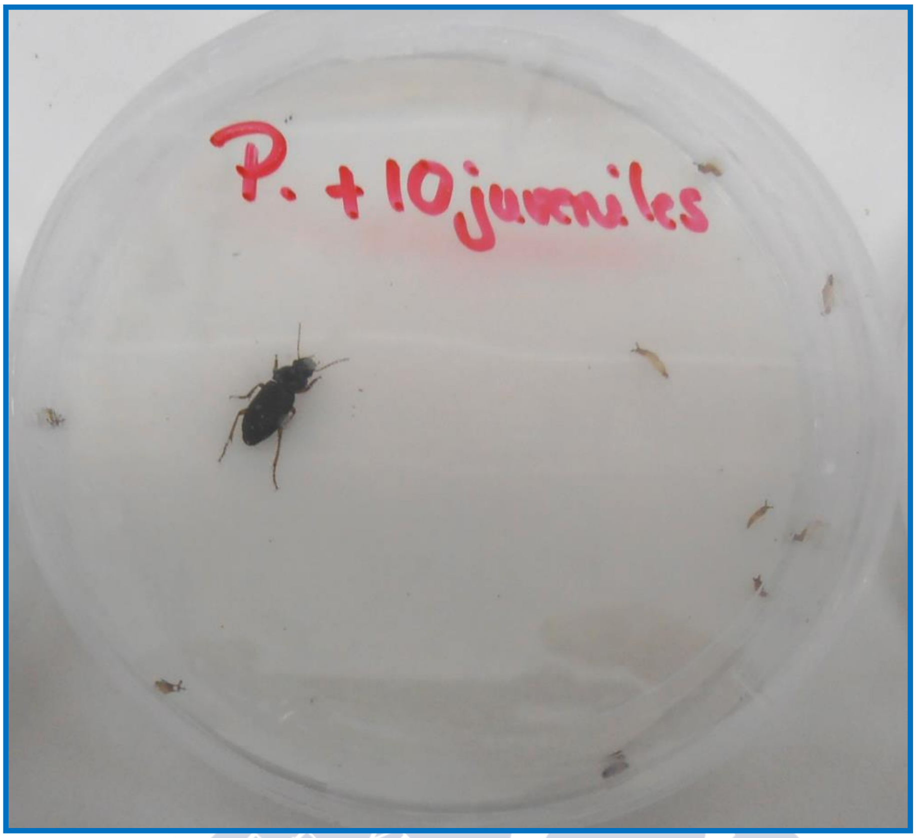

CHAPTER 2

El-Danasoury H., Cerecedo C., Córdoba M., Iglesias-Piñeiro J. (2016) Predation by the carabid beetle *Harpalus rufipes* on the pest slug *Deroceras reticulatum* in the laboratory. *Annals of Applied Biology*, doi:10.1111/aab12337.

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Predation by the carabid beetle *Harpalus rufipes* on the pest slug *Deroceras reticulatum* in the laboratory

H. El-Danasoury, C. Cerecedo, M. Córdoba, J. Iglesias-Piñeiro

Abstract

The Harpalini species *Harpalus rufipes*, as many other generalist carabids, consume a wide variety of prey and it is known to feed on pest slugs such as the grey field slug *Deroceras reticulatum*, but quantitative data about the predatory activity of *H. rufipes* on slugs are very scarce. In laboratory experiments, we assessed the capability of male *H. rufipes* to kill eggs and different-sized slugs of the pest species *D. reticulatum* in either the absence or the presence of alternative live prey (dipteran larvae and aphids). We also investigated the preference of *H. rufipes* for eggs and hatchlings of *D. reticulatum* in a choice experiment. *H. rufipes* killed considerable amounts of eggs and small juveniles (≤5.0 mg) of *D. reticulatum*, both in no-choice and in choice situations. Medium-sized juvenile slugs (10–20 mg) were seldom killed only in no-choice situations, and no large juveniles (50–60 mg) were killed. Dipteran larvae and aphids were killed also in no-choice and in choice situations. The type of alternative prey presented with slug eggs affected the survival of the eggs to *H. rufipes* predation. The presence of dipteran larvae as alternative prey did not affect the survival of juvenile slugs. When eggs and small juvenile slugs were offered together, the survivals of both items were similar. The obtained results under laboratory conditions suggest that the generalist predator *H. rufipes* might realise an important contribution to the control of pest slugs.

Keywords: alternative prey, biological control, *Deroceras reticulatum*, *Harpalus rufipes*, slug eggs, slug predator, slug size.

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CHAPTER 3

El-Danasoury H., Iglesias-Piñeiro J. (2017) Predation by polyphagous carabid beetles on eggs of the pest slug *Deroceras reticulatum*: effect of temperature and potential implications of climate change.
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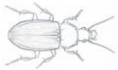

Predation by polyphagous carabid beetles on eggs of the pest slug *Deroceras reticulatum*: effect of temperature and potential implications of climate change

Heba El-Danasoury and Javier Iglesias-Piñeiro

Departamento de Zooloxía, Xenética e Antropoloxía Física, Facultade de Bioloxía, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain

Running tittle: Predation of carabids on eggs of a pest slug

Keywords *Harpalus rufipes*, *Poecilus cupreus*, *Deroceras reticulatum*, slug eggs, predation, temperature response, biological control, climate change, egg density.

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Abstract

1 It is expected that climate change and global warming will have a dramatic impact on ectothermic animals. Terrestrial slugs are pests of increasing importance in temperate and tropical regions, and polyphagous carabids which are slug predators are being considered as promising biological control agents through conservation biological control. *Harpalus rufipes* and *Poecilus cupreus* are two polyphagous carabids which are abundant and widespread in agroecosystems of the temperate Northern hemisphere and which are known to destroy eggs of the pest slug *Deroceras reticulatum* in the laboratory.

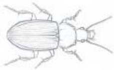
2 To examine the effect of temperature on the predatory activity of *H. rufipes* and *P. cupreus* on the eggs of *D. reticulatum* a laboratory experiment with different constant temperatures, and an outdoor experiment in semi-field conditions under current ambient temperature and under a simulated warmed-up-climate, were performed.

3 In both experiments *H. rufipes* killed more eggs than *P. cupreus* and was the unique species whose predatory activity significantly increased with increasing temperature.

4 To our knowledge, this is the first study on the predatory activity of polyphagous carabids on the eggs of a pest slug performed out of the laboratory under semi-field conditions and covering a realistic predicted climate warming scenario. Results suggest that biological pest control performed by polyphagous carabids such as *H. rufipes* upon pest slugs may be enhanced under predicted climate warming conditions.

Introduction

The warming of Earth as a consequence of climate change and their impacts on ecosystems on all continents is undeniable (IPCC, 2014). In Europe, the expected warming is greatest over the eastern countries during winter, and over western and southern Europe in spring-summer; thus, projected increase in summer temperatures could exceed 6 ˚C in parts of France and the Iberian Peninsula by the end of the 21st century under some emission scenarios (Olesen *et al.*, 2011).



For ectothermic animals temperature is probably the most important environmental factor affecting their biology (Taylor, 1981; Brown *et al.*, 2004). Therefore, it is expected that global warming will have a dramatic impact on these animals, and during the last years multiple works have shown that climate change affect their distribution (Kocmánková *et al.*, 2011; Capinha *et al.*, 2014), behaviour (Dalin, 2011; Hoffmann & Sgrò, 2011) and/or dynamics (Willis *et al.*, 2006; Altermatt, 2010), among other aspects of their biology.

Although often overlooked, terrestrial gastropods (slugs and snails) are pests of increasing importance in temperate and tropical regions due to the impact they have on a vast array of crops over a wide geographical range, and because they are implicated in the transmission of many plant pathogens as well as parasites of humans, domestic livestock and wild mammals (South, 1992; Barker, 2002). The grey field slug *Deroceras reticulatum* (Müller) is the most widespread slug species worldwide and is believed to be responsible for most of the slug damage in economic terms (Speiser, 2002). Slug control is mainly based in the use of pelleted molluscicides, which operate only in the short-term and whose performance depends on weather conditions (Bailey, 2002); moreover, they may affect non-target organisms negatively, including natural enemies of gastropods and other pests (Langan *et al.*, 2004). The only commercial biological control agent for gastropods is the rhabtitid nematode *Phasmarhabditis hermaphrodita* (Wilson *et al.*, 1993), but the high cost of the biocontrol product based on it limits its use to high value horticultural crops (Glen & Wilson, 1997; Moens & Glen, 2002). Under these circumstances and the general increase of environmental concern and demand for food quality by consumers, integrated pest management of slugs is highly desirable, including the optimization of the regulation exercised by natural enemies.

Carabids beetles are widely recognized as beneficial organisms in agroecosystems and are increasingly regarded as crucial for regulating many pest populations as some synthetic pesticides are phased out (Kromp, 1999; Sunderland, 2002; Symondson *et al.*, 2002; Berthe *et al.*, 2015). It is known that many polyphagous beetles within the Carabidae are important gastropod predators and they are the major natural enemies that are being considered as biological control agents of slugs as an alternative to commercial molluscicides, particularly through conservation biological control (Symondson, 2004). Carabids are mainly ground-dwelling predators (Kromp, 1999) and a number of species predate on slug eggs (Ayre, 2001; Oberholzer & Frank, 2003; Hatteland *et al.*, 2010; Pianezzola *et al.*, 2013), which are laid very superficially on the soil (Oberholzer & Frank, 2003). The amount of viable eggs is a key factor in the population dynamics of slugs (Heller, 2001; Schley & Bees, 2002), and thus carabid species which kill slug eggs in the field may make a significant contribution to the control of populations.

Harpalus rufipes (De Geer) and *Poecilus cupreus* (Linnaeus) are two polyphagous carabids abundant and widespread in agroecosystems of the temperate Northern hemisphere (Kromp, 1999), which are known to destroy eggs of *D. reticulatum* in the laboratory (Oberholzer & Frank, 2003; El-Danasoury *et al.*, 2016). It is also known that temperature has a positive effect on the predatory activity of these and others generalist carabids when preying on live, moving prey, in the laboratory (Kielty *et al.*, 1999; Ayre, 2001; Frank & Brambröck, 2016), but predation on slug eggs is much less documented. Therefore, the main purposes of the present study were to examine the effect of temperature on the predatory activity *H. rufipes* and *P. cupreus* on the eggs of *D. reticulatum* and to gain insight into their ability to kill slug eggs under more natural conditions. For this, we performed a laboratory experiment to compare the amounts of eggs killed by the beetles at 5 different constant temperatures over 24 h, and an outdoor experiment in semi-natural conditions to compare the amounts of eggs killed over 72 h under current ambient temperature and under a simulated warmed-up-climate with increased temperature; in the latter experiment we tested also two different egg densities.

Materials and methods

Test organisms

Beetles were collected from April 2016 in a grassy uncultivated field (42º 48’ 33’’ N; 8º 38’ 12’’ W) near Santiago de Compostela by pitfall traps baited with a slightly moist mixture of wheat bran and sugar. Twenty traps were set in the field the week before the start of each experiment, during which time the traps were checked and emptied every morning. In the laboratory, the *H. rufipes* and *P. cupreus* individuals were selected and classified by species, sex and date of collection. Subsequently they were kept without food in transparent plastic boxes with perforated walls and lids, with the floor covered with wet filter paper and refuges consisting of small pieces of black plastic tubes. The beetles were kept in a climate room at 16 ºC with a 8 dark : 16 light photoperiod. Only male beetles were used in the experiments, since females were infrequently caught. They were used just once and always after they had remained between four and eight days in starvation after they were collected.

Slug eggs were obtained in the laboratory from field collected adults *D. reticulatum*. Slugs were hand collected at night in the same field as the beetles at approximately monthly intervals from February 2016, and were kept in the same climate room and boxes as the beetles, but with food consisting of pieces lettuce, carrots and cabbage. The boxes and food were replaced twice weekly and inspection for eggs was performed every day. The eggs were collected and kept on wet filter paper in the darkness at 16 ºC, inside Petri dishes labelled with the date.

The entire course of the development of the embryo of *D. reticulatum* is observable when the egg is immersed in water and viewed under transmitted light (Carrick, 1938). About ten days after collection, the eggs were inspected under a binocular microscope for the selection of those to be used in the experiments. Only eggs containing a single living embryo and without foreign inclusions were selected, while non-motile embryos were considered to be dead (Iglesias *et al.*, 2000).

Eggs of approximately the same age were used in each of the experiments. Killed eggs were considered those which lost their round shape and looked like smashed remains of the egg shell (Pianezzola *et al.*, 2013). The eggs recovered at the end of the experiments were examined under a binocular microscope, as described above, to check whether they were still alive or not. Eggs which completely disappeared were considered as entirely eaten by the beetles.

Egg predation at different temperatures in the laboratory

The amounts of eggs killed by the two beetle species at five different constant temperatures were assessed using incubators (Radiber AGP500HR, Barcelona, Spain) maintained at 12, 14, 16, 18 or 20 ˚C, with a 8 dark : 16 light photoperiod. Experimental units consisted of 9-cm diameter petri dishes with the bottom lined with wet filter paper, a small piece of black plastic tubing acting as shelter for the beetle, and 20 live eggs of *D. reticulatum* placed in a group in the centre of each dish to emulate the way that eggs can be found in the field. For each temperature, 12 petri dishes were used, 5 of which had one *H. rufipes* each, 5 had one *P. cupreus* each, and 2 served as controls with eggs but without beetle. The beetles used for each temperature were individually weighed to the nearest 0.1 mg with a scale Sartorius BP211D, and then allowed to adapt to the respective temperature by keeping them 24 h in the proper incubator inside petri dishes with wet filter paper and shelter. After that, at the middle point of the photo-phase, the beetles were added to test dishes with eggs and the control dishes were placed in the incubators. After 24 h the beetles were removed and the eggs of each dish were collected and examined to check whether they were alive or not.

Egg predation in semi-natural conditions

An experiment was performed outdoor to compare the amounts of eggs killed by the two beetle species under two different climate conditions and densities of eggs. The experiment was performed within 14 brick-built plots measuring 1 × 0.5 m and 20 cm height walls each. The plots were arranged in 2 parallel rows of seven plots situated next to the laboratory and oriented south-easterly. The whole area of the plots was covered with a transparent roof to protect them against rain.

Experimental units consisted of plastic trays measuring 44 × 28 × 8 cm with the bottom holed to allow drainage and lined with a double layer of filter paper (FILTER-LAB® RM13055858), which was filled with a 4 cm layer of moistened soil. The soil was a field collected loam soil (36.3% sand, 43.9% silt, 19.8% clay, 2.8% organic matter and pH 6.7) which had been previously heated in an oven at 105 ˚C for 24 h to eliminate any predators, pests or parasites potentially present, allowed to cool at room temperature and strained through a 2 mm sieve. The inner walls of the trays over the soil level were smeared with an aqueous dispersion of polytetrafluoroethylene (Fluon® AD309E, Whitford Ltd, Cheshire, UK) in order to prevent the escape of the beetles. Live slug eggs were placed on the soil surface in groups of five eggs. In order to emulate field conditions more closely, the whole tray surface was covered with a layer of senescent leaves of chestnut tree collected in the same field as the beetles and adult slugs; the leaves had been kept frozen for 6 months, allowed to defrost at room temperature and washed with tap water. The position of the eggs was signalized by means of toothpicks stuck in the leaves and soil.

The factors used in the experiment were (i) climate conditions, with levels current-climate and warmed-up-climate; (ii) egg density, with levels low and high density, and (iii) beetle species, with levels *H. rufipes* and *P. cupreus*.

The current-climate conditions were represented by 7 plots which were just covered with a plastic netting of 1 × 1 cm mesh to avoid birds. The warmed-up-climate conditions were represented by 7 plots which were covered with a lid made of a transparent plastic sheet, to produce a greenhouse effect. Air temperature and humidity during the experiment were monitored at hourly intervals by means of 2 automatic data-takers HOBO® Pro v2 (Onset Computer Corporation, Bourne, Massachusetts, USA) placed each at 5 cm height within one of the plots used for each climate conditions. The low and high egg densities consisted of four or eight groups of 5 eggs respectively, evenly placed on the soil surface of the trays.

Two trays were placed in each plot. Both the plots corresponding to each climatic condition and the trays corresponding to each combination of beetle species × egg density were distributed at random. Thus, there were three replicated trays for every combination of climate × egg density × beetle species. One control tray without beetles was used for each of the four combinations of climate × egg density.

The experiment was run for 3 days between 21 and 24 June 2016. The first day, once the trays were ready, two previously weighed beetles of the same species were released in each tray at noon. Each day, at sunset, the trays were watered to keep moist the leaves and the soil; to avoid mess up and watering of the chemical barrier of the trays, the water was carefully applied to the surface of the trays using a thin plastic tube to pour the water directly on the leaves, avoiding any splash effect; water was applied at a rate of 200 mL per day and tray.

After 72 h, the beetles were carefully searched for and removed from the trays. Then the eggs of each tray were collected and examined to check whether they were alive or not.

Statistical analyses

Data on weights of beetles were $\log_{10}$ transformed, and data on the proportion of live eggs at the end of the experiments were arcsine transformed to stabilize the variance prior to analysis. The analyses were performed using the IBM SPSS Statistics package (Version 19, 2010; IBM Corporation, Somer, NY, USA), by means of univariate general linear models with the different factors studied in the experiments introduced as fixed factors. Data in the text are shown as mean ± SE and sample size (*N*).

Results

Egg predation at different temperatures in the laboratory

The weights of the beetles used in the experiment were 92.4 ± 1.9 mg in the case of *H. rufipes* and 77.3 ± 1.5 mg in the case of *P. cupreus* ($N = 25$). The weights of the two species were significantly different but they were similar across temperatures and both factors did not interact (beetle species ANOVA: $F_{1,40} = 40.15$, $P < 0.001$; temperature ANOVA: $F_{4,40} = 1.38$; $P = 0.26$; beetle × temperature ANOVA: $F_{4,40} = 1.07$; $P = 0.39$).

At the end of the experiment all the beetles and control eggs were alive, and all test dishes contained live eggs. Overall, in 24 h one *H. rufipes* killed 9.04 ± 0.83 (range 3-17) eggs of *D. reticulatum* and *P. cupreus* 4.44 ± 0.51 eggs (range 0-10) ($N = 25$). Both beetle species and temperature had significant effects on the proportion of live eggs after 24 h exposition to the beetles, but the two factors did not interact (beetle species ANOVA: $F_{1,40} = 39.18$; temperature ANOVA: $F_{4,40} = 9.91$; $P < 0.001$; beetle × temperature ANOVA: $F_{4,40} = 1.42$; $P = 0.25$); the proportion of live eggs after 24 h was lower for eggs exposed to *H. rufipes* than for eggs exposed to *P. cupreus*, but only for *H. rufipes* significantly decreased with increasing temperature (Fig. 1).

Egg predation in semi-natural conditions

The weight of the beetles used in the experiment were 100.8 ± 1.7 mg in the case of *H. rufipes* and 84.4 ± 1.3 mg in the case of *P. cupreus* ($N = 24$). The weights of the two species were significantly different but they were similar across climate conditions and egg densities (beetle species ANOVA: $F_{1,40} = 58.53$, $P < 0.001$; climate conditions ANOVA: $F_{1,40} = 0.09$, $P = 0.76$; egg density ANOVA: $F_{1,40} = 1.33$, $P = 0.26$), and all interactions between factors were non-significant ($P > 0.05$ in all cases).

Table 1 summarizes the values of temperature and relative humidity recorded in the plots over the experiment. In the warmed-up-climate plots, mean temperature was nearly 3.0 ˚C higher than in the current-climate plots. In both climate conditions the mean relative humidity was higher than 80%, but it was slightly lower in the current-climate plots.

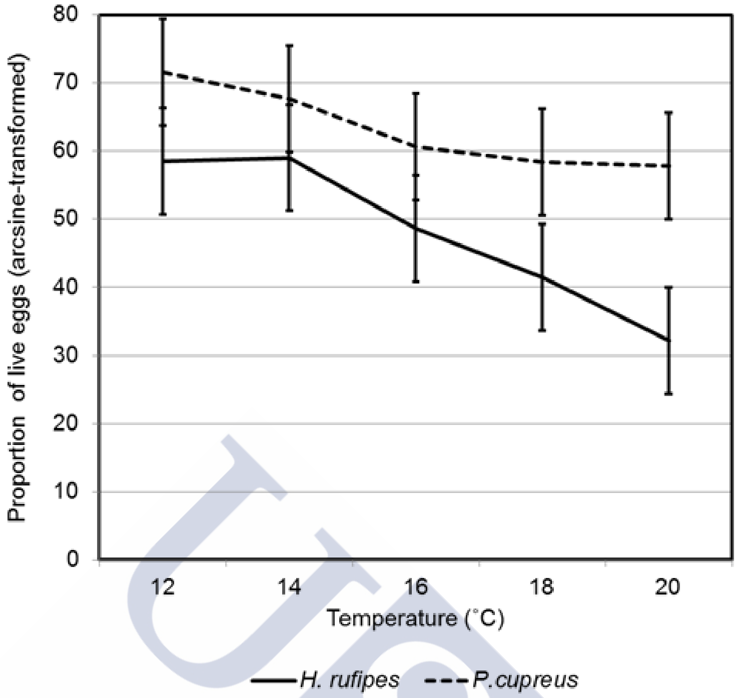


Figure 1 Proportion of live eggs of *Deroceras reticulatum* after 24 h exposition to either one *Harpalus rufipes* or one *Poecilus cupreus* in the laboratory at five different constant temperatures. Data shown are arcsine transformed means with 95% confidence limits of 5 replicated dishes.

Table 1 Temperature and relative humidity (RH) recorded for the two climate conditions in the experimental plots. Values shown are means ± SD (*N* = 72), and minimum / maximum values (in brackets).

	Climate conditions	
	Current-climate	Warmed-up-climate
Temperature (ºC)	18.6 ± 3.1 (14.3 / 26.1)	21.5 ± 4.4 (15.5 / 31.3)
RH (%)	83.5 ± 9.9 (65.0 / 95.1)	87.5 ± 8.8 (66.4 / 96.5)

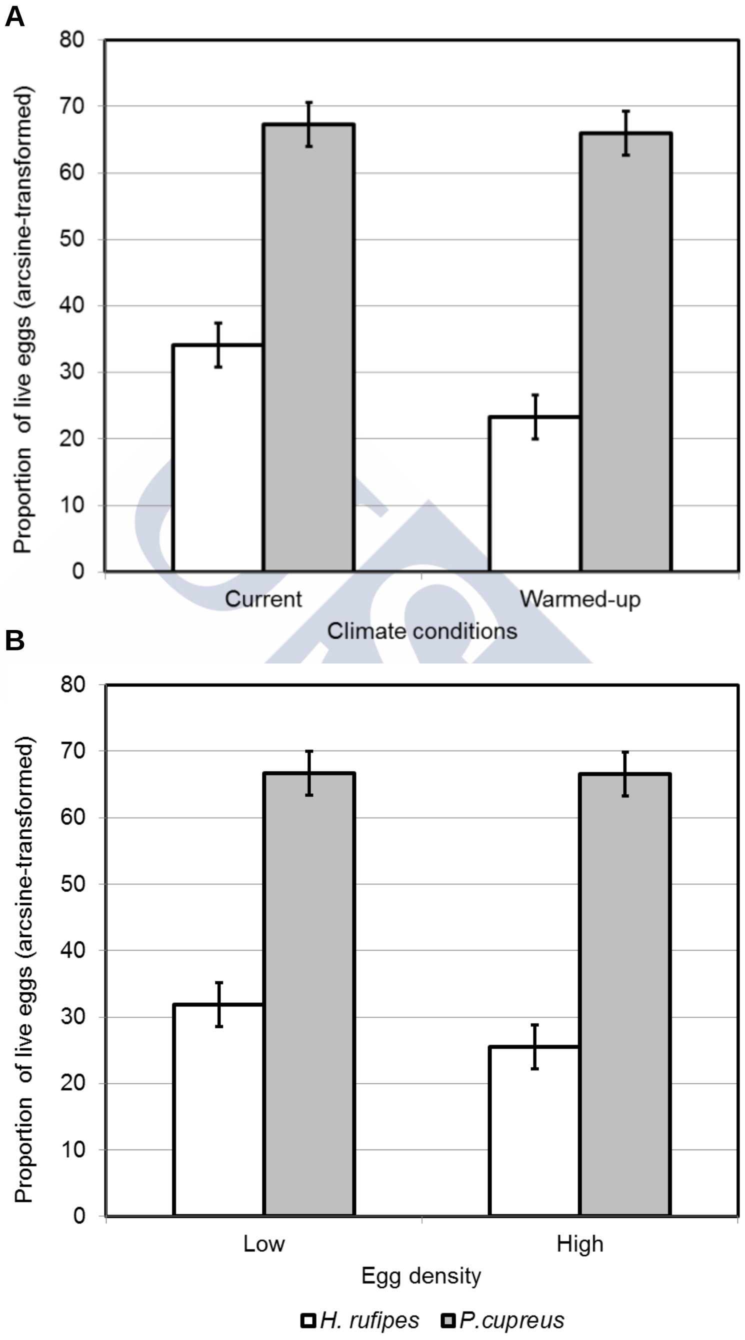


Figure 2 Proportion of live eggs of *Deroceras reticulatum* after 72 h exposition to either two *Harpalus rufipes* or two *Poecilus cupreus* in the experiment performed outdoor, according to (A) climate conditions and (B) egg density. Data shown are arcsine transformed means with 95% confidence limits of 6 replicated trays.

At the end of the experiment all beetles could be recovered from the test trays and all contained live eggs. No mortality was observed in the control eggs. Beetle species and climate conditions had both significant effects on the proportion of live eggs after 72 h exposition to the beetles, and the two factors showed a significant interaction (beetle species ANOVA: $F_{1,16} = 587.78$, $P < 0.001$; climate conditions ANOVA: $F_{1,16} = 15.08$, $P < 0.01$; beetle × climate ANOVA: $F_{1,16} = 8.95$, $P < 0.01$); the survival of the eggs was lower when exposed to *H. rufipes*, and only for this species it was significantly lower under warmed-up than under current-climate conditions (Fig. 2A). Egg density, and the interaction beetle species × egg density, showed both marginally significant effects (egg density ANOVA: $F_{1,16} = 4.22$, $P = 0.057$; beetle × egg density ANOVA: $F_{1,16} = 3.99$, $P = 0.063$) (Fig. 2B); there was a trend to lower egg survival at high egg density, and this was due to *H. rufipes*.

Discussion

The reported experiments support previous findings about the ability of the polyphagous beetles *H. rufipes* and *P. cupreus* to kill eggs of the pest slug *D. reticulatum* in petri dishes in the laboratory. In similar no-choice laboratory experiments, Oberholzer & Frank (2003) found a range of 1-10 eggs of *D. reticulatum* destroyed in 24 h by one *P. cupreus* maintained with a temperature cycle of 15 ˚C for 12 h and 10 ˚C for 12 h; Al-Danasoury *et al.* (2016) reported ranges of 6-11 and 7-13 eggs killed in 24 h by one *H. rufipes* in two different experiments performed at 16 ˚C; in the laboratory experiment reported here, *P. cupreus* destroyed eggs in the range 0-10 and *H. rufipes* in the range 3-17 when all temperatures were pooled. In the outdoor experiment, two *H. rufipes* killed between 65% and 89% of the eggs in 72 h, while the figures for two *P. cupreus* were between 15% and 17%; to our knowledge, this is the first demonstration of the ability of both carabid species to find and kill eggs of *D. reticulatum* outside petri dishes, under emulated field conditions.

The survival of *D. reticulatum* eggs exposed to *H. rufipes* decreased with increasing temperatures, both in the laboratory and in the outdoor experiment, which agrees with previous findings pointing a positive impact of temperature on the predatory activity of generalist carabid beetles. In laboratory experiments, Kielty *et al.* (1999) found a positive relationship between temperature and the number of aphids predated by *H. rufipes* and by

Pterostichus madidus, while Ayre (2001) found a positive relationship between temperature and the proportions of *H. rufipes* or *P. madidus* individuals predating on newly-hatched *D. reticulatum*. Recently, Frank & Bramböck (2016) showed that four polyphagous carabid species abundantly found in spring in European arable crops (*Amara ovata*, *Harpalus distinguendus*, *Poecilus cupreus* and *Anchomenus dorsalis*) killed more larvae of the pollen beetle *Meligethes aeneus* at rising temperature, demonstrating for the first time a higher predatory activity of polyphagous carabids on an insect pest under realistic forecasted climate warming scenarios, with increases of mean temperatures of 3 and 5 ˚C over current mean temperatures of their region (Eastern Austria). The outdoor experiment reported here studied the predatory activity of polyphagous carabids on slug eggs under a mean temperature increased 3˚ over current mean temperature, a warming scenario which falls within the temperature increases predicted for NW-Spain for the second half of the century (De Castro *et al.*, 2005; Martínez de la Torre & Míguez-Macho, 2009). Overall, all these findings suggest that biological pest control exercised by different species of polyphagous carabids upon different pest prey may be enhanced under climate warming conditions.

As predicted by the metabolic theory of ecology for ectothermic animals (Brown *et al.*, 2004), temperature-mediated increases of metabolic rates and therefore of demand for food are expected with increasing temperature; in turn, higher demand for food implies higher foraging activity and higher overall activity, which has been recorded for carabids in field studies (Honek, 1997; Tuf *et al.*, 2012; Berthe *et al.*, 2015). Although these arguments can help to explain the results obtained in this and other studies, there are other factors which have to be considered, such as the particular traits of predators and prey. In the work of Frank & Bramböck (2016) *P. cupreus* was the species which killed the highest number of *M. auneus* larvae and showed an increased predatory activity at higher temperature. However, in the present work, the predatory activity of *P. cupreus* upon the eggs of *D. reticulatum* was not significantly affected by temperature neither in the laboratory nor in the outdoor experiment. In the laboratory experiments of Oberholzer & Frank (2003), *P. cupreus* killed less eggs of *D. reticulatum* than *Pterotichus melanarius* when the eggs were offered alone; when the eggs of *D. reticulatum* were offered with alternative prey, the amount destroyed by *P. cupreus* was significantly reduced, while alternative prey did not affect *P. melanarius* predation on the eggs of *D. reticulatum*; *P. cupreus* also killed less slugs than *P. melanarius* both in the absence and in the presence of alternative prey. Oberholzer *et al.* (2003) showed that *P.*

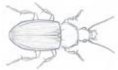
cupreus failed to reduce damage to germinating oilseed rape caused by *D. reticulatum*, while *P. melanarius* significantly reduced slug damage caused by this slug species. However, *P. cupreus* and *Anchomenus dorsalis* were observed to reduce populations of oilseed rape insect pests in winter rape fields (Zaller *et al.*, 2009). These and other findings (Symondson, 2004) suggest that terrestrial molluscs are non-attractive prey for *P. cupreus*, while it has much more potential as antagonist of insect pests, and this could explain why *P. cupreus* showed a significant increased predatory activity on *M. auneus* larvae at higher temperature (Frank & Bramböck, 2016), but in the present work the predatory activity of *P. cupreus* upon the eggs of *D. reticulatum* was unaffected by temperature.

The same interspecific differences regarding the effect of temperature on the predatory activity of these beetles upon the eggs of *D. reticulatum* were observed regarding the density of egg prey. A positive effect of prey density on the amount of prey killed by *H. rufipes* has been reported for predation on adult *Myzus persicae* aphids (Loughridge & Luff, 1983) or on larvae of the medfly *Ceratitis capitata* (Monzó *et al.*, 2011), and with *P. cupreus* for predation on *Rhopalosiphum padi* aphids or on fruit flies or house crickets (Lang & Gsödl, 2003). However, in our outdoor experiment, only *H. rufipes* showed a trend to lower egg survival at higher egg density, which may be explained as well by the bare willingness of *P. cupreus* to predate on slug eggs.

Not surprisingly, egg mortality was proportionally lower in the outdoor than in the laboratory experiment, mainly in the case of *P. cupreus*; the same was observed by Hatteland *et al.* (2010) when comparing predation of *P. melanarius* or *Pterostichus niger* on eggs and hatchlings of *Arion vulgaris* (often regarded as *A. lusitanicus*) in laboratory and semi-field experiments, reflecting that higher available area and environmental heterogeneity demand a higher and more realistic prey-searching effort from the beetles than the experiments in petri dishes. Moreover, Hatteland *at al.* (2010) found that while *P. melanarius* and *P. niger* attacked similar proportions of eggs and hatchlings of *A. vulgaris* when each item was offered alone or when both items were offered together in petri dishes, they killed much higher proportions of eggs than of hatchlings when both items were offered together under semi-field conditions. This reflects that small slugs are more likely to escape predation under field conditions because they have access to a great number and diversity of refugia, as shown by McKemey *et al.* (2003), while slug eggs by no means can escape predation if attacked. Al-

Danasoury *et al.* (2016) did not find differences in the survival of eggs and hatchling of *D. reticulatum* exposed to *H. rufipes* in choice experiments in petri dishes. Besides, predation on slug eggs by polyphagous carabids may be less constrained by beetle size than predation on slugs, because small carabid species which are incapable of overcoming the defences (thickness of body wall and ability to produce defensive mucus) of even the smallest slug, could potentially affect their population dynamics by preying on slug eggs (Symondson, 2004). Positive relationships between the size of the beetle species and their ability to predate on slugs had been pointed from both laboratory (Ayre, 2001) and field studies (Tod, 1973; Ayre & Port, 1996), as well as negative relationships between slug size and their susceptibility to be killed by generalist carabid beetles (Paill, 2000, 2004; McKemey *et al.*, 2001; Oberholzer & Frank, 2003; Hatteland *et al.*, 2010, 2011; Al-Danasoury *et al.*, 2016). However, we do not know whether there exists any relationship between size of the beetle predator and size and/or toughness of slug eggs, although some observations suggest that such relationship may exist. Carabid beetles can easily break the slug eggshell (Pianezzola *et al.*, 2013), and it has been recorded that even small species such as *Nebria brevicollis*, an oligophagous (Seric Jelaska *et al.*, 2014) beetle of similar size to *P. cupreous* (Homburg *et al.*, 2013), was able to destroy the relatively hard eggs of *A. vulgaris* in the laboratory, although it was unable to kill newly-hatched slugs (Hatteland *et al.*, 2010). The eggs of *A. vulgaris*, which are larger and harder (tougher shell) than those of *D. reticulatum*, are less susceptible to be destroyed by *P. melanarius* in both no-choice and choice situations (Oberholzer & Frank, 2003). Experiments with different prey species and slug eggs are needed to confirm the existence of this relationship.

While some species of ground beetles are able to actively search for slugs by detecting their mucus trails (Digweed, 1994; Thomas *et al.*, 2008; McKemey *et al.*, 2004), it is not known whether the predators actively search for slug eggs or they are randomly found. Even if beetles kill slug eggs by finding them at random, their ability to aggregate in areas of high slug density, as found in field studies (Symondson *et al.*, 1996; Bohan *et al.*, 2000) will increase the probability of these highly mobile predators to find and kill slug eggs. Furthermore, the obtained results suggest that under predicted climate warming conditions it is expected that carabids will show higher foraging activity, which in turn will increase their probabilities to find and destroy slug eggs.



A consistent finding of studies on the predatory activity of carabids upon a number of different prey is that beetles do not consume all that they kill (Hagley *et al.*, 1982; Symondson, 1989; Ayre, 2001; Lang & Gsödl, 2003; Hatteland *at al.*, 2010; Al-Danasoury *et al.*, 2016). That superfluous or wasteful killing is regarded as a beneficial component of the impact that carabids may have on pest organisms (Ayre, 2001; Lang & Gsödl, 2003). However, such assassin behaviour likely is strongly affected by experimental conditions such as reduced space, absence of refugia or high availability of even non-attractive prey, thus research is clearly needed to confirm whether the same behaviour of carabids persists under field conditions or not.

Both *H. rufipes* and *P. cupreus* are considered as typical representatives of the carabid fauna of open habitats (Tuf *et al.*, 2012) and agricultural land (Fusser *et al.*, 2016). In Europe, *H. rufipes* is usually considered an autumn breeding species, while *P. cupreus* is considered a spring breeder (Tuf *et al.*, 2012). However, Den Boer & Den Boer-Daanje (1990) showed that the division of Western European carabids in spring or autumn breeders, or in adult or larval hibernators, are inadequate, and that the activity period of most species usually extends over most of the year. In particular, adults of most Harpalini species are active during most months of the year, although are generally less active during cooler months (Kromp, 1999; Larochelle & Larivière, 2005). In Northumberland (England), Luff (1980) found that adult *H. rufipes* were active from April to November. In the Czech Republic, Honek (1997) reported peaks of abundance in spring and early summer for *P. cupreus* and in late summer for *H. rufipes*. In North Spain, Miñarro & Dapena (2003) found adult *H. rufipes* and *P. cupreus* from August to October in apple orchards, although they did not sample other months. Langmaack *et al.* (2001) collected adult *H. rufipes* and *P. cupreus* in oil seed rape crops in spring-summer in Northern Germany. Fusser *et al.* (2016) sampled semi-natural habitats in a number of locations in agricultural landscapes in Southwest Germany in spring-summer and found *H. rufipes* and *P. cupreus* as the most abundant out of 128 carabid species. El-Danasoury *et al.* (2016) collected adult *H. rufipes* from the end of April to the beginning of July in northwest Spain in 2015, and in the present work we collected adults of both carabid species from April to June 2016.

Breeding in *D. reticulatum* takes place whenever environmental conditions are suitable (Carrick 1938; South 1989), leading to both geographical and inter-annual variability in the breeding periods and the pattern of generations (South, 1992). Previous field studies at different localities in our region point to the presence of eggs of *D. reticulatum* throughout most of the year (Barrada *et al.*, 2004; Córdoba *et al.*, 2011) and thus, in our region, the beetles will have high probabilities to find eggs of *D. reticulatum* in the field. Furthermore, Berthe *et al.* (2015) found that *H. rufipes* was the coleopteran species which showed the highest increase in activity-density in response to a 2 ˚C artificial warming of a spring-shown wheat crop.

To our knowledge, this is the first study on the predatory activity of polyphagous carabids on the eggs of a pest slug performed outdoors under semi-field conditions and covering a realistic predicted climate warming scenario. This and previous studies clearly suggest that biological pest control performed by polyphagous carabids upon pest prey may be enhanced under predicted climate warming conditions. However, forecasting the responses of organisms and ecological relationships between organisms to future climatic conditions is difficult, because climate change may also affect different aspects of the biology of prey, of predators and their natural enemies, the quality and/or quantity of available food, and many other factors. In the agricultural context, climate change is expected to affect also the varieties and species of crops grown, as well as the timing of agricultural practices, among other factors which might affect any particular organism and its relationships with the biotic and abiotic environment.

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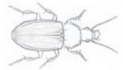

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CHAPTER 4

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Performance of the slug parasitic nematode *Phasmarhabditis hermaphrodita* under predicted conditions of winter warming

Heba EL-DANASOURY and Javier IGLESIAS-PIÑEIRO

Abstract

The nematode *Phasmarhabditis hermaphrodita* is the only commercial biological control agent for terrestrial slugs. We investigated whether the predicted conditions of winter warming could have any effect on its performance. In the presence of nematodes, slug damage to lettuce plants and slug survival were significantly lower under the predicted conditions of winter warming than under normal winter conditions, while in the absence of nematodes, slug damage and survival were similar under the conditions of winter warming and under current winter conditions. The data suggest that *P. hermaphrodita* may perform better under the predicted conditions of winter warming.

Keywords: *Phasmarhabditis hermaphrodita*, *Deroceras reticulatum*, winter warming, climate change, nematodes, slugs.

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GENERAL DISCUSSION


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GENERAL DISCUSSION

Unwanted pests have hindered human efforts in land management since the onset of organized agriculture in the Neolithic. Thus, pest management has been an integral aspect of human civilization, and has evolved from simple manual techniques to sophisticated strategies employing pheromones, biopesticides or genetically modified organisms (GMOs). Without doubt, pest management has played a role of paramount importance in the impressive rise in food production in the last 50 years (Oerke 2006). Currently, the most rational and successful strategy is considered to be integrated pest management (IPM), which include the integrated implementation of cultural, mechanical, chemical, and biological control measures. Even so, the application of chemicals remains the most widely used method for pest control worldwide (Ziska and McConnell, 2016), although increasing awareness of health and environmental issues, as well as increasingly restrictive legislation on pesticide use in developed countries, pose a great challenge to future agriculture, as reliance on pesticides must be drastically reduced (Hossard *et al.*, 2014; Lamichhane *et al.*, 2015). Concomitantly, global food production must increase by 50 % to meet the projected demand of the world's population by 2050 (Chakraborty and Newton, 2011; Foley *et al.*, 2011). In essence, there is an urgent need to meet food demands by increasing crop yield and satisfy sustainability goals by reducing reliance on pesticides, which is challenging task for twenty-first century agriculture, further complicated against the background of climate change.

There is a general agreement in that climate change will affect pest biology due to two elementary relationships. First, increased global temperature will have profound effects in ectotherm organisms such as most agricultural pests, altering their metabolic rates and demand for food, competitive ability, spread, phenology and population dynamics, as have already been reported. Second, increased atmospheric CO_2 means higher availability of an essential resource for plants, that currently lack optimal levels of CO_2 needed to maximize photosynthesis, growth and reproduction (Wang *et al.*, 2012), and therefore pests and pathogens will indirectly be affected through their crop hosts (Ziska and Runion, 2007). Overall, the multiple expected effects of climate change on the biology of agricultural pests converge on an increased pest pressure, and this turns unclear if previous management paradigms will be adequate in the future (Ziska and McConnell, 2016).

Increased pest pressure is expected to result in increased pesticide use (Ziska, 2014), in the form of higher amounts, doses, frequencies and range of varieties or types of products applied. At the same time, it is also expected that climate change will affect the efficacy of chemical pesticides, for a number of reasons including reduced application opportunities, lower effectiveness of application methods, reduced pesticide efficacy due to accelerated degradation, and increased injury of non-target organisms (Delcour *et al.*, 2015). Both rising temperatures and increased pesticide use are likely to increase pesticide resistance in pest organisms due to augmented exposure and evolutionary rate of genetically different strains under warm conditions (Jackson *et al.*, 2011; Delcour *et al.*, 2015). Although the most evident responses of pest organisms to climate change are phenology alterations and/or geographical redistributions (Scherm, 2004), there is also a high likelihood that climate change, especially warming, will exacerbate the already high phenotypic plasticity of most pest species, and even the appearance of genetic adaptation of pests to new climate conditions (Bloomfield *et al.*, 2006; Lepetz *et al.*, 2009; Lamichhane *et al.*, 2015).

Among terrestrial gastropods, there exist a number of examples of local adaptations attributed to high ecophysiological plasticity (Wolda and Kreulen, 1973; Briner and Frank, 1998; Martin and Bergey, 2013; Slotsbo *et al.*, 2013). In this work, significant changes in the feeding pressure exerted by *D. reticulatum* populations under predicted climatic conditions of winter warming and increased summer rainfall, have been found, even when the experiments were run for only seven days (Chapter 1). The obtained results suggest a higher potential of the species for crop damaging under predicted climatic conditions. The outcome of these experiments agrees with known traits of *D. reticulatum*, such as its wide-ranging tolerance to environmental factors and high adaptability to unstable environments (Runham and Hunter, 1970; Port and Port, 1986; South, 1992; Willis *et al.*, 2008). Indeed, *D. reticulatum* currently inhabits a very high diversity of climates types, and although ecophysiological plasticity can help to explain this fact, just its great ability to search and exploit suitable microenvironments and microclimates, which in turn increases their likelihood to thrive in a wide diversity of macroclimatic conditions, undoubtedly makes a great contribution to explain its wide geographic and climatic range (Capinha et *al.*, 2014). In short, *D. reticulatum,* as many other invasive pest species, is very likely to successfully adapt to future climatic conditions and to increase its potential for crop damaging.

With the expectation of increased pest pressure and increased resistance to pesticides in pest organisms, biological control does not only turn up as an environment friendly option, but also as a solution with high probabilities of success in keeping pest populations below acceptable thresholds, both for already established pests and to potential invasive new pest species (Delcour *et al.*, 2015; Lamichhane *et al.*, 2015). Therefore, biological control is a priority area of research which will help to adapt agriculture to the changing climatic conditions and, under this view, pro-active studies devoted to identify natural enemies as possible biocontrol agents of pest species are essential (Jackson *et al.*, 2011). In this context, generalist natural enemies, i.e., natural enemies able to attack a wide range of prey including those already established as well as exotic and invasive ones, are seen as the most promising biocontrol agents of pests (Lu *et al.*, 2012; Liu *et al.*, 2014).

Here, it has been shown that a previously overlooked polyphagous carabid beetle, *Harpalus rufipes*, which is one of the most common and abundant ground beetle in a wide range of agroecosystems in most European countries and in the USA (Harrison and Gallandt, 2012), may realise an important contribution to the control of *D. reticulatum* populations by predating upon its eggs and small juveniles (Chapter 2). In laboratory experiments, *H. rufipes* was able to kill considerable amounts of eggs and small juveniles of *D. reticulatum*, both when they were the only available prey, and when the beetles had a choice between slugs or eggs and alternative prey known to be predated by *H. rufipes* in the field, such as live aphids or live dipteran larvae. Moreover, *H. rufipes* was able to find and kill eggs of *D. reticultum* in outdoor experiments emulating field conditions, and showed a significant increase in predatory activity with increasing temperature, both in the laboratory and in the outdoor experiment (Chapter 3); *H. rufipes* also showed a trend to higher predatory activity with higher egg density. The smaller polyphagous carabid beetle *Poecilus cupreus* was also able to destroy eggs of *D. reticultum* in the laboratory, and to find and kill eggs under semi-field conditions, but the predatory activity of *P. cupreus* on slug eggs was always lower than that of *H. rufipes*, and it was unaffected neither by temperature nor by egg density; our results and those of previous studies suggest that *P. cupreus* has a high potential as antagonist of insect pests, but terrestrial slugs and their eggs seems to be non-attractive prey for *P. cupreus*.

An outstanding finding consistently observed in studies about the predatory activity of carabid beetles upon a number of different prey, is that beetles do not consume all that they kill, and this seemingly superfluous killing is regarded as an important beneficial component of the impact that carabids have on pest organisms. This phenomenon has been recorded by many authors, and also by us, in different species of beetles predating upon a number of different prey in laboratory and semi-field conditions. However, such behaviour is likely to be strongly affected by experimental conditions, and its existence under field conditions guarantees further research.

Another important finding is that slug eggs seem to be more susceptible than slugs (even the smallest slugs) to predation by generalist beetles, especially when environmental heterogeneity demands a prey-searching effort from the beetles. This can be explained because slugs are more likely to escape predation by sheltering, as they have access to a great number and diversity of refugia under field conditions, while slug eggs by no means can escape predation if attacked. Besides, predation on slug eggs by beetles should be less constrained by beetle size than predation on slugs, since small beetle species which are incapable to subdue even the smallest slugs, have been shown to be able to destroy slug eggs (Hatteland *et al.*, 2010). This is important because the amount of viable eggs is a key factor in the population dynamics of pest slugs (Heller, 2001; Schley and Bees, 2002), and therefore beetles capable of killing eggs are expected to make a significant contribution to the control of populations.

Abundance, a long period of seasonal activity, and circadian rhythm coincidence with the prey are important attributes of a generalist predator to significantly contribute to the control of a pest (Symondson *et al.*, 2002; Winder *et al.*, 2005). Available data suggest that *H. rufipes* meet all those criteria with regard to *D. reticulatum* and, in the particular case of Galicia, this beetle will have high probabilities to find eggs and hatchlings of *D. reticulatum* both at the beginning (April-June) and at the end (October-November) of its activity period, and therefore high probabilities to cause a considerable impact on populations of *D. reticulatum*.

A point of concern about the necessary synchrony between pest species and natural enemies is that, as a result of climate change, the phenology of biocontrol agent and pest target may be differentially affected (Scherm, 2004; Gutierrez *et al.*, 2008; Ziska and McConnell, 2016). Fortunately, generalist natural enemies such as many carabid beetles are usually highly adaptable species (Holland and Luff, 2000), and *H. rufipes*, in particular, has been found to increase abundance under warming conditions. Buchholz *et al.* (2013), in a simulated drought experiment (i.e. warming of a grassland community of arthropods), found a disproportionately increased abundance of *H.rufipes* and an overall reduced beetle diversity. Similarly, Berthe *et al.* (2015) found that *H. rufipes* was the beetle species which showed the highest increase in activity-density in response to a 2 ˚C artificial warming of a spring-shown wheat crop, while beetle diversity decreased. Together, the results of Buchholz *et al.* (2013) and those of Berthe *et al.* (2015) suggest that climate warming promotes common and highly adaptable beetle species at the cost of others, resulting in an overall loss of beetle diversity. From the point of view of biological control, climate-driven increases in the activity-densities of generalist beetle species such as *H. rufipes*, are expected to enhance the important service that these natural enemies provide to agroecosystems controlling major agricultural pests (Holland and Luff, 2000).

The effect of predicted conditions of winter warming on the performance of the rhabditid nematode *Phasmarhabditis hermaphrodita*, as slug biocontrol agent, was studied in Chapter 4. *P. hermaphrodita* is the only commercial biological control agent for terrestrial gastropods, but it is known that is common and widespread in many European countries, especially in central and north Europe, where it is believed to perform a natural control of susceptible slug species (Rae *et al.*, 2007). It is also known that *P. hermaphrodita* is very sensitive to moderately high temperatures (Maupas, 1900), and therefore is suspected to be highly susceptible to global warming. Recently, Wilson *et al.* (2015) studied the effect of temperature on the interaction between *P. hermaphrodita* and *D. reticulatum*, in the range of the slug’s optimum temperature, which is 14 °C, and up to 24 °C; they found that the nematode is much less tolerant of increasing temperatures than the host slug, so that when parasites were present, slug feeding increased significantly with raising temperatures as the inhibitory effect of the parasites lessened, but in the absence of parasites, slug feeding significantly decreased over the same range as temperature rose above the slug's optimum; Wilson *et al.* (2015) concluded from their findings that rising temperatures due to global warming will increase slug problems in the European home range of the slug and the nematode, as *P. hermaphrodita* will perform worse in controlling slug damage. However, high levels of abundance of *D. reticultum* and of damage caused by its feeding activity are common, widespread, and well known over the autumn-winter in many European countries, with much lower temperatures than those used by Wilson *et al.* (2015); in fact, crops such as winter wheat or winter rape are among the most damaged by *D. reticulatum* and other slugs (Port and Port, 1986; South, 1992).

Here, we performed an experiment under semi-natural conditions to study the effect of winter warming on the interaction between *P. hermaphrodita* and *D. reticulatum*, comparing its performance under normal (non-manipulated) winter temperature (mean 9.7 ˚C), and under winter warming conditions with a rise in mean temperature of 4.5 ˚C, which matches the rise in winter temperature predicted for the last third of the century in north-west Spain (De Castro *et al.*, 2005). Performance was measured as damage to lettuce plants and as slug survival. We found that, in the presence of nematodes and under predicted winter temperatures, slug damage to lettuce plants and slug survival were lower than under normal winter temperatures, while in the absence of nematodes slug damage and survival were similar under under normal and predicted winter temperatures. These findings suggest that increased mean winter temperature in the range 4.0-4.5 ˚C, as predicted for NW-Spain, should improve the performance of *P. hermaphrodita* as slug biocontrol agent during the winter.

The commercial *P. hermaphrodita* is reared in monoxenic culture with the bacterium *Moraxella osloensis*, which produces an endotoxin consisting of a lipopolysaccharide lethal to slugs (Tan and Grewal, 2002). However, *P. hermaphrodita* is a facultative parasite known to be capable of growing on a wide range of bacteria (Wilson *et al.*, 1995), and when living saprobically *P. hermaphrodita* associates with complex and variable bacterial assemblages while keeping unchanged its virulence to *D. reticulatum* (Rae *et al.*, 2010). Thus, as suggested by Tan and Grewal (2001), *P. hermaphrodita* is expected to persist in the environment living saprobically on decaying hosts, slug faeces or leaf litter, and therefore may be suitable for long-term inoculative slug control, since inundative applications are unfeasible from the economic point of view. According to our results, in areas where climate change brought winter temperatures nearer to the optimum of both parasite and host, as expected for NW-Spain, the performance of both naturally occurring and commercial *P. hermaphrodita* as slug biocontrol agent should be expected to improve. As a consequence, important crops severely damaged by slugs, such as winter wheat or winter rape, may benefit from natural control exerted by wild nematodes and/or from long-term inoculative slug control.

Crop damage by pests is the result of complex ecological dynamics between two or more organisms, and is therefore very difficult to predict (Rosenzweig *et al.*, 2001; Scherm, 2004). As discussed above, forecasting is much more intricate with the background of climate change. Globalization and climate change are leading to an international homogenization of pest threats (Ziska and McConnell, 2016), and although a great body of research on potential consequences of climate change on agriculture already exists, our level of uncertainty, and our inability to make confident predictions is such, that studies in this regard can be considered to remain in their infancy (Lamichhane *et al.*, 2015). Models, which are perceived as essential tools to support decision making and guide research needs, should be taken with caution because of their high level of uncertainty (Chakraborty, 2013; Delcour *et al.*, 2015), and this highlights the urgent need for more research on several fronts. Improved biological control appears as one of the most promising strategies (Jackson *et al.*, 2011), but its success depends on the acquisition of in-depth knowledge about pests, natural enemies, their interactions and their relationships with the biotic and abiotic environment (Lamichhane *et al.*, 2015). In this context of uncertainty, simulation studies like those presented here provide valuable insights and information for posterior modelling studies.


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CONCLUSIONS


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CONCLUSIONS

1. Predicted climatic conditions of winter warming and increased summer rainfall caused an increase in the feeding pressure exerted by *Deroceras reticulatum* populations on cultivated plants. Given its recognised great adaptability to environments and climates, as well as its wide distribution, it should be expected an increase in pest problems caused by this species in the future.

2. The polyphagous carabid beetle *Harpalus rufipes* is able to find and kill eggs and small juveniles of *D. reticulatum*, and its predatory activity increases with increasing temperature. Given that *H. rufipes* is one of the most common and abundant ground beetles in European agroecosystems, and that is known to respond to predicted rise in temperature by increasing its activity-density, it should be expected that *H. rufipes* plays a significant role as antagonist of *D. reticulatum* under future climatic conditions.

3. Slug eggs are more susceptible than even the smallest slugs, to predation by generalist beetles such as *H. rufipes* and *Poecilus cupreus*. This means that beetles capable of killing eggs may make a significant contribution to the control of slug pest populations, but predation on slug eggs is difficult to reveal and deserves further research.

4. A rise of nearly 4.5 ˚C in mean winter temperature enhanced the effectiveness of the commercial strain of *Phasmarhabditis hermaphrodita* in controlling damage caused by *D. reticulatum* populations. Given that *P. hermaphrodita* is a facultative parasite able to live saprobically on a wide range of bacteria while keeping its virulence to *D. reticulatum,* and that this nematode is common and abundant in European soils, it should be expected an improvement in the performance as slug biocontrol agent of both the commercial strain and the naturally occurring *P. hermaphrodita*, in areas

where climate change brings winter temperatures closer to the 14 ˚C optimum temperature of both the nematode and its slug host.

5. Measures that promote abundance of natural enemies such as generalist carabid beetles should be implemented and assessed, as biocontrol services provided by natural enemies are regarded as one of the most promising strategies to fight pest in the climate change scenario.

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SUMMARY


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SUMMARY

Keywords: pest slugs, climate change, biological control, *Deroceras reticulatum*, carabid beetles, parasitic nematodes, slug eggs.

Changes in climate have caused significant impacts on different ecosystems on all continents in recent decades. Climate models for Europe predict significant warming, changes in the rainfall pattern and an increase in the frequency of extreme weather events. As temperature is considered the most dominant environmental factor affecting the biology of ectotherms, it is expected that global warming will have a deep impact on their population dynamics, leading to invasions and spread of pests and diseases able to survive under warmer climatic conditions. Over the last decade, multiple reports have shown that climate change is already altering the distribution, behaviour, and/or population dynamics of many agricultural pests around the globe. As a consequence, there is growing concern that global food security is threatened by the emergence and/or spread of crop pests and pathogens.

Terrestrial gastropods can cause severe damage to crops, can act as vectors of transmission of plant pathogens, and as intermediary hosts of parasites of domestic and wild animals. A number of species of terrestrial slugs and snails are considered important agricultural pests because they cause serious damage to plants cultivated by man, affecting a wide variety of species and productive sectors, from large intensive crops to public and private gardens, including horticulture, floriculture, forestry, fruit growing, grassland and commercial crops of ornamental, aromatic and medicinal plants. While some land snails can reach pest status even in relatively arid regions, slugs are especially problematic in temperate and rainy climates. Several slug species of the genus *Deroceras* have been dispersed almost worldwide through human activities, and this is particularly true in the case of *Deroceras reticulatum* (Müller, 1774), a successful opportunistic and invasive species considered the most serious slug pest worldwide, that is currently found in most temperate and subtropical regions, including Europe, Asia, Australia, New Zealand, Tasmania, Canada, the United States, South America, South Africa and many islands of the Pacific and Atlantic Oceans.

The most common method of slug control is the use of chemical molluscicides, which are usually pellets or baits containing between 2% and 8% of either metaldehyde or methiocarb as active ingredient. Both molluscicidal compounds have negative impacts on non-target organism, such as carabid beetles, which in turn are important predators of terrestrial gastropods, and domestic and wild animals, especially birds and mammals. A chemical molluscicide containing iron phosphate as active ingredient is sold under the claims of being natural and safe to wildlife and domestic animals, but recent research have shown that molluscicide pellets containing iron phosphate have adverse effects on earthworm survival, activity and growth, and present significant hazards to other soil inhabiting invertebrates, domestic animals and human child.

Regarding natural enemies, slugs and snails are preyed upon by a wide range of both vertebrates and invertebrates, and are parasitized by a number of dipterans, nematodes and mites. It is unlikely that most of these natural enemies could perform an effective control of populations of pest gastropods in the field, but research showed that carabid beetles and nematodes are promising biological control agents. In fact, the only biological control agent ever marketed for terrestrial gastropods is the nematode *Phasmarhabditis hermaphrodita* (Schneider, 1859).

Climate change will not only affect pest species of concern, but will also affect the biology of their natural enemies, the quality and/or quantity of available food, and many other factors. Thus, it is becoming more and more evident that modelling projections of responses to climate change by any particular organism should take into account not only the particular species, but also their natural enemies and ecological relationship. To be accurate, models need to be parameterised and validated by experimental evidences, such as experimental manipulations of temperature and precipitation, which have already provided valuable information into the responses of different components of terrestrial ecosystems to predicted climate change. This is a fundamental previous step for modelling the functional response of agro-ecosystems to climate change at the farm-level or wider scales. Modelling studies have suggested that the abundance, range, and intensity of damage caused by *D. reticulatum* may increase in coming years in Europe due to the effects of climate change, but these models do not account for factors other than population dynamics and physiological traits of the own species.

The main objective of this PhD work is to explore potential changes in the feeding behaviour of the pest slug *Deroceras reticulatum*, as well as in the behaviours of some of their most important natural enemies, under climatic conditions predicted for Galicia by the last third of the century. The studied natural enemies were the carabid beetles *Harpalus rufipes* (De Geer, 1774) and *Poecilus cupreus* (Linnaeus, 1758), and the slug parasitic nematode *Phasmarhabditis hermaphrodita*. In order to achieve this general objective, different experiments were performed under laboratory controlled conditions and under semi-natural conditions.

Chapter 1 reports two experiments designed to assess the behavioural response of *D. reticulatum* to different climate manipulations in terms of herbivory, by measuring the damage inflicted by slug populations to lettuce seedlings. The experiments were performed outdoors under semi-natural conditions. The climate manipulations tested emulate predicted climatic conditions for Northwest Spain, specifically winter warming and increased summer rainfall, in contrast with normal winter conditions and summer without rain conditions, respectively. In the winter experiment, a normal winter treatment was compared with a winter warming treatment, with higher temperature, lower relative humidity and the absence of rainfall. In the summer experiment, a summer without rain treatment was compared with an increased summer rainfall treatment, characterised by the presence of rainfall, whereas the conditions of temperature and relative humidity were similar in both summer treatments. The results showed significant changes in the feeding pressure exerted by *D. reticulatum* under predicted climatic conditions, pointing to a higher potential of the species for crop damaging.

Chapter 2 reports a series of laboratory experiments designed to assess the capability of the carabid beetle *H. rufipes* to kill eggs and different-sized *D. reticulatum* slugs, either in the absence or in the presence of alternative prey. While it was known that many polyphagous carabid beetles are important gastropod predators, most studies have focused on just a few species of beetles. *H. rufipes*, however, received little attention even when is one of the most common and abundant ground beetle in a wide range of agroecosystems in most European countries. The results showed that *H. rufipes* is able to kill considerable amounts of eggs and small juveniles of *D. reticulatum*, both in no-choice and in choice situations, suggesting that this beetle might realise an important contribution to the control of *D. reticulatum* populations.

Chapter 3 reports two experiments, one performed under laboratory controlled conditions and one under semi-natural conditions, designed to examine the effect of temperature on the predatory activity of the carabid beetles *H. rufipes* and *P. cupreus* on the eggs of *D. reticulatum*. The laboratory experiment tested five different constant temperatures, and the outdoor experiment compared current ambient temperature in spring-summer with an experimentally manipulated higher temperature emulating predicted warming conditions. In both experiments, *H. rufipes* killed more eggs than *P. cupreus*, and was the unique species whose predatory activity significantly increased with increasing temperature, suggesting that biological pest control performed by *H. rufipes* upon *D. reticulatum* may be enhanced under predicted climate warming conditions.

Chapter 4 reports an outdoor experiment comparing the performance of the slug biocontrol agent nematode *P. hermaphrodita*, under normal winter conditions and under winter warming conditions emulating predicted warmer winters. In the presence of nematodes, slug damage to lettuce plants and slug survival were significantly lower under the predicted conditions of winter warming than under normal winter conditions, while in the absence of nematodes, slug damage and survival were similar under the conditions of winter warming and under current winter conditions. Thus, the results suggest that *P. hermaphrodita*, as biological control agent against *D. reticulatum*, may perform better under predicted conditions of winter warming.

Overall, from the obtained results it should be expected that both the pest slug *D. reticulatum* and its natural enemies *H. rufipes* and *P. hermaphrodita* will be affected by predicted conditions of climate. Specifically, it should be expected a higher potential of *D. reticulatum* populations for crop damaging, and an enhanced performance of *H. rufipes* and *P. hermaphrodita* as biological control agents of *D. reticulatum*. In turn, these findings emphasize the importance of taking into account natural enemies and ecological interactions when modelling the response of species to climate change.

RESUMEN


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RESUMEN

Palabras clave: plagas de babosas, cambio climático, control biológico, *Deroceras reticulatum*, nematodos parásitos, huevos de babosas.

Los cambios en el clima han causado impactos significativos en diferentes ecosistemas de todos los continentes a lo largo de las últimas décadas. Los modelos climáticos para Europa predicen un calentamiento significativo, cambios en el patrón temporal y espacial de distribución de las lluvias, y un aumento en la frecuencia de fenómenos meteorológicos extremos. La temperatura se considera el factor ambiental más importante en la biología de los organismos ectotermos, por lo cual se espera que el calentamiento global tenga un profundo impacto en su dinámica poblacional, lo que conduce a esperar invasiones y aumentos del rango de distribución de organismos causantes de plagas y enfermedades que sean capaces de sobrevivir bajo condiciones climáticas más cálidas. Durante la última década, numerosas investigaciones han demostrado que el cambio climático ya está alterando la distribución, el comportamiento y/o la dinámica poblacional de muchas plagas agrícolas en todo el mundo. Como consecuencia, existe una preocupación creciente con respecto a que la seguridad alimentaria mundial esté amenazada por la aparición y/o propagación de plagas y patógenos de los cultivos.

Los gasterópodos terrestres pueden causar graves daños a los cultivos, pueden actuar como vectores de transmisión de patógenos de las plantas, y también como hospedadores intermediarios de parásitos de animales domésticos y silvestres. Varias especies de babosas y caracoles terrestres son consideradas plagas agrícolas de gran importancia porque pueden causar serios daños a las plantas cultivadas por el hombre, afectando a una amplia variedad de especies y sectores productivos, desde los grandes cultivos intensivos hasta jardines públicos o privados, incluyendo la horticultura, floricultura, silvicultura, fruticultura, pastos y cultivos comerciales de plantas ornamentales, aromáticas y medicinales. Algunos caracoles terrestres pueden alcanzar el estatus de plagas incluso en regiones relativamente áridas, pero las babosas son especialmente problemáticas en climas templados y lluviosos. Varias especies de babosas del género *Deroceras* se han dispersado por casi todo el mundo gracias al hombre, y esto es

particularmente cierto en el caso de *Deroceras reticulatum* (Müller, 1774), una exitosa especie oportunista e invasora, considerada como la babosa causante de plagas más perjudicial, a nivel mundial. Actualmente *D. reticulatum* se encuentra en la mayoría de las regiones templadas y subtropicales, incluyendo Europa, Asia, Australia, Nueva Zelanda, Tasmania, Canadá, Estados Unidos, Sudamérica, Sudáfrica y muchas islas de los océanos Pacífico y Atlántico.

El método más común para el control de las babosas es el uso de molusquicidas químicos, que habitualmente son pellets o cebos que contienen como ingrediente activo entre un 2% y un 8% de metaldehído o de metiocarbamato. Ambos compuestos molusquicidas tienen impactos negativos sobre otros organismos, como escarabajos de la familia Carabidae, que a su vez son importantes depredadores de gasterópodos terrestres en el campo, y también sobre animales domésticos y silvestres, especialmente aves y mamíferos. De forma relativamente reciente han comenzado a comercializarse cebos molusquicidas que contienen fosfato de hierro como ingrediente activo, y que reivindican ser productos totalmente naturales y seguros para la fauna y los animales domésticos. Sin embargo, recientes investigaciones han demostrado que los molusquicidas con fosfato de hierro tienen también efectos adversos sobre la fauna; en particular se ha demostrado que afectan a la supervivencia, crecimiento y actividad de lombrices terrestres, y existen indicios de que también representan un peligro para otros invertebrados del suelo, así como para animales domésticos y para humanos, especialmente niños.

Con respecto a los enemigos naturales, las babosas y los caracoles son depredados por una amplia gama de vertebrados e invertebrados, y son parasitados por numerosas especies de dípteros, nematodos y ácaros. En el caso de la mayoría de estos enemigos naturales, es poco probable que puedan realizar un control efectivo en el campo de las poblaciones de gasterópodos perjudiciales, pero numerosas investigaciones han demostrado que los carábidos y los nematodos presentan un elevado potencial como agentes de control biológico de gasterópodos. De hecho, el único agente de control biológico de gasterópodos que se comercializa es el nematodo *Phasmarhabditis hermaphrodita* (Schneider, 1859).

Una de las consecuencias del cambio climático global es un aumento de la expectativa de mayores problemas ocasionados por las plagas agrícolas, así como de un incremento en el desarrollo de resistencia a los plaguicidas por parte de muchas especies dañinas. Ante estas expectativas, el control biológico de plagas aparece no sólo como una opción no agresiva para el medio ambiente, sino también como una alternativa con elevadas probabilidades de éxito para mantener las poblaciones de organismos causantes de plagas por debajo de umbrales aceptables, tanto en el caso de plagas ya establecidas como en el caso de potenciales nuevas especies invasoras. Desde este punto de vista, el control biológico de plagas se convierte en un objetivo prioritario de investigación, con el fin de contribuir a adaptar la agricultura a las condiciones climáticas cambiantes. Una primera fase, esencial, la representan los estudios dedicados a la identificación de enemigos naturales que puedan ser utilizados como agentes de control biológico de plagas. Los conocimientos actuales en este ámbito indican que los enemigos naturales generalistas, es decir, aquellos capaces de atacar a una amplia gama de presas, son los más prometedores como agentes de control biológico de plagas en un ambiente cambiante.

Es evidente que el cambio climático no sólo afecta a las especies causantes de plagas, sino también a sus enemigos naturales, a la calidad y/o cantidad de alimento disponible para ellas, y a muchos otros factores. En función de esto, las proyecciones realizadas por los modelos de predicción de las respuestas al cambio climático por parte de cualquier organismo deben tener en cuenta, no sólo a las especies de interés en particular, sino también a sus enemigos naturales y sus relaciones ecológicas. El éxito de los modelos depende en gran medida de los parámetros que utilicen y de su validación por medio de evidencias experimentales, tales como manipulaciones experimentales de la temperatura y la precipitación, las cuales ya han proporcionado valiosa información sobre las respuestas de diferentes componentes de los ecosistemas terrestres al cambio climático. Éste es un paso previo fundamental para la modelización de la respuesta funcional de los agroecosistemas al cambio climático, no sólo a nivel de especie sino a escalas más amplias. Estudios previos de modelización sugieren que la abundancia, el rango de distribución, y la intensidad de los daños causados por *D. reticulatum* pueden aumentar en los próximos años en Europa debido a los efectos del cambio climático, pero dichos modelos sólo han tenido en cuenta las propiedades fisiológicas y las relacionadas con la dinámica poblacional de la propia especie.

El objetivo principal de este trabajo de doctorado es explorar potenciales modificaciones en el comportamiento alimentario de la babosa *Deroceras reticulatum*, así como en el comportamientos de algunos de sus enemigos naturales más importantes, bajo condiciones climáticas pronosticadas para Galicia en el último tercio del presente siglo. Los enemigos naturales estudiados fueron los escarabajos *Harpalus rufipes* (De Geer, 1774) y *Poecilus cupreus* (Linnaeus, 1758), y el nematodo zooparásito *Phasmarhabditis hermaphrodita*. Para alcanzar este objetivo general se realizaron diferentes experimentos bajo condiciones controladas en el laboratorio, y bajo condiciones semi-naturales.

En el Capítulo 1 se presentan dos experimentos diseñados para evaluar la respuesta de *D. reticulatum* frente a diferentes manipulaciones climáticas, en términos de consumo de plantas cultivadas. Para ello se cuantificó el daño infligido por poblaciones de babosas a plántulas de lechuga. Estos experimentos se realizaron al aire libre bajo condiciones semi-naturales. Las manipulaciones climáticas que se utilizaron emulan condiciones climáticas previstas para el Noroeste de España, en concreto el calentamiento invernal y el aumento de precipitación en verano. En el experimento de invierno se comparó un tratamiento que representa un invierno normal, con un tratamiento de calentamiento invernal, caracterizado por temperaturas más altas, menor humedad relativa y ausencia de lluvia. En el experimento de verano se comparó un tratamiento que representa un verano normal sin lluvia, con un tratamiento de verano con precipitación, caracterizado por la presencia de lluvias, mientras que las condiciones de temperatura y humedad relativa fueron similares en ambos tratamientos de verano. Los resultados mostraron cambios significativos en la cantidad de daño ocasionado a las plantas por *D. reticulatum* bajo las condiciones climáticas pronosticadas, lo cual sugiere un incremento del potencial de esta babosa para causar daños a los cultivos en el futuro.

En el Capítulo 2 se presenta una serie de experimentos de laboratorio diseñados para valorar la capacidad del escarabajo *H. rufipes* para depredar sobre huevos y sobre babosas de diferentes tamaños, de la especie *D. reticulatum*, tanto en ausencia como en presencia de presas alternativas. Es bien conocido que muchas especies de escarabajos polífagos son importantes depredadores de gasterópodos, pero la mayoría de los estudios realizados hasta la actualidad se han centrado en sólo unas pocas especies de escarabajos. *H. rufipes*, sin embargo, ha recibido poca atención, aun cuando es uno de los escarabajos más comunes y abundantes en todo tipo de agroecosistemas en la mayoría de los países europeos. Los resultados demostraron que *H. rufipes* es capaz de matar considerables cantidades de huevos y de pequeños juveniles de *D. reticulatum*, tanto en situaciones en las que no existe alternativa como en situaciones con otras presas disponibles. Esto sugiere que *H. rufipes* podría realizar una importante contribución al control de las poblaciones de *D. reticulatum*.

En el Capítulo 3 se describen dos experimentos, uno realizado bajo condiciones controladas de laboratorio, y otro bajo condiciones semi-naturales, diseñados para estudiar el efecto de la temperatura sobre la actividad depredadora de los escarabajos *H. rufipes* y *P. cupreus* sobre los huevos de *D. reticulatum*. En el experimento del laboratorio se utilizaron cinco temperaturas constantes diferentes, y en el experimento al aire libre se comparó la actual temperatura ambiente de primavera-verano con una temperatura manipulada experimentalmente para emular las condiciones previstas de calentamiento. En ambos experimentos *H. rufipes* destruyó más huevos que *P. cupreus* y también fue la única especie cuya actividad depredadora aumentó significativamente con el aumento de la temperatura, lo que sugiere que el control biológico realizado por *H. rufipes* sobre *D. reticulatum* puede verse favorecido bajo las condiciones previstas de calentamiento.

En el Capítulo 4 se compara la eficacia como agente de control biológico de *D. reticulatum* del nematodo *P. hermaphrodita*, bajo condiciones invernales actuales y bajo condiciones de calentamiento invernal. En presencia del nematodo, tanto el daño causado por las babosas a plantas de lechuga como la supervivencia de las babosas fueron significativamente menores bajo las condiciones de calentamiento invernal. En ausencia de nematodos, el daño causado por las babosas y su supervivencia fueron similares en ambas condiciones. Por lo tanto, los resultados sugieren que la eficacia de *P. hermaphrodita* como agente de control biológico de *D. reticulatum* puede aumentar en las condiciones previstas de calentamiento.

Globalmente, los resultados obtenidos indican que tanto la babosa *D. reticulatum*, como sus enemigos naturales *H. rufipes* y *P. hermaphrodita*, se verán significativamente afectados por las condiciones climáticas previstas. En concreto, los resultados señalan que sería de esperar una mayor capacidad de las poblaciones de *D. reticulatum* para ocasionar daños a los cultivos, y una mayor eficacia de *H. rufipes* y *P. hermaphrodita* para actuar como agentes de control biológico de esta especie de babosa. Estos hallazgos enfatizan la importancia de considerar a los enemigos naturales y las interacciones ecológicas entre especies a la hora de modelizar la respuesta de las especies a futuras condiciones climáticas.

Del presente trabajo de investigación se han extraído las siguientes conclusiones generales:

1. Las condiciones climáticas previstas de calentamiento invernal y de aumento de las precipitaciones en verano provocaron un aumento en el daño causado por la actividad alimentaria de poblaciones de *Deroceras reticulatum* a plantas cultivadas. Dada su gran adaptabilidad a diversos ambientes y climas, así como su amplia distribución geográfica, en el futuro cabe esperar un aumento en los perjuicios causados por esta especie de babosa a la producción agrícola.

2. El escarabajo polífago *Harpalus rufipes* es capaz de localizar y matar huevos y pequeños juveniles de *D. reticulatum,* y su actividad depredadora sobre estas presas aumenta si aumenta la temperatura. Dado que *H. rufipes* es uno de los escarabajos terrestres más comunes y abundantes en los agroecosistemas europeos, y dado que responde a los incrementos de temperatura previstos para el futuro aumentando su abundancia y actividad, cabe esperar que *H. rufipes* desempeñe un importante papel como antagonista natural de *D. reticulatum* en condiciones climáticas futuras.

3. Los huevos de las babosa son más susceptibles que las propias babosas, incluídos los juveniles más pequeños, a la depredación por parte de escarabajos generalistas como *H. rufipes* y *Poecilus cupreus*. Esto implica que las especies de escarabajos capaces de destruir los huevos de las babosas pueden realizar una significativa contribución al control de las poblaciones de babosa causantes de plagas. Sin embargo, la existencia de depredación sobre los huevos de las babosas por parte de sus enemigos naturales es difícil de poner de manifiesto, de manera que este tipo de depredación debe de ser objeto de mayor investigación.

4. Un incremento de aproximadamente 4.5 ˚C en la temperatura media de invierno resultó en un aumento de la eficacia de la cepa comercial de *Phasmarhabditis hermaphrodita* como agente de control biológico de *D. reticulatum*. Dado que *P. hermaphrodita* es un parásito facultativo, capaz de vivir como saprobionte asociándose a una amplia gama de bacterias, pero manteniendo su virulencia para *D. reticulatum*, y dado que este nematodo es común y abundante en los suelos de los agroecosistemas europeos, en zonas en las que el cambio climático provoque un aumento de las temperaturas invernales de magnitud tal que éstas se aproximen a los 14 ˚C, cabe esperar un aumento de la eficacia de *P. hermaphrodita* como antagonista de *D. reticulatum*, y este aumento de eficacia debería ser aplicable tanto de la cepa comercial como a las poblaciones naturales de *P. hermaphrodita*.

5. En las zonas de producción agrícola deberían implementarse, y evaluarse, medidas destinadas a favorecer el establecimiento y la proliferación de poblaciones de escarabajos generalistas y otros enemigos naturales, de las babosas, y de muchas otras plagas, puesto que una de las estrategias más prometedoras para combatir las plagas agrícolas en el contexto del cambio climático, es fomentar y aprovechar al máximo el servicio que proporcionan los enemigos naturales para el control de plagas.

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RESUMO


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RESUMO

Palabras chave: pragas de lesmas, cambio climático, control biolóxico, *Deroceras reticulatum*, nematodos parasitos, ovos das lesmas.

Os cambios no clima teñen causado impactos significativos en diferentes ecosistemas de todos os continentes ao longo das últimas décadas. Os modelos climáticos para Europa prevén un quecemento significativo, cambios no patrón temporal e espacial de distribución das choivas, e un aumento na frecuencia de fenómenos meteorolóxicos extremos. A temperatura considérase o factor ambiental máis importante na bioloxía dos organismos ectotermos, polo cal se espera que o quecemento global teña un profundo impacto na súa dinámica poboacional, o que conduce a esperar invasións e aumentos do rango de distribución de organismos causantes de pragas e enfermidades que sexan capaces de sobrevivir baixo condicións climáticas máis cálidas. Durante a última década, numerosas investigacións demostraron que o cambio climático xa está a alterar a distribución, o comportamento e/ou a dinámica poboacional de moitas pragas agrícolas en todo o mundo. Como consecuencia, existe unha preocupación crecente con respecto a que a seguridade alimentaria mundial estea ameazada pola aparición e/ou propagación de pragas e patóxenos dos cultivos.

Os gasterópodos terrestres poden causar graves danos aos cultivos, poden actuar como axentes de transmisión de patóxenos das plantas, e tamén como hóspedes intermediarios de parasitos de animais domésticos e silvestres. Varias especies de lesmas e caracois terrestres considéranse pragas agrícolas de importancia xa que poden causar serios danos ás plantas cultivadas polo home, afectando a unha ampla variedade de especies e sectores produtivos, dende os grandes cultivos intensivos, ata xardíns públicos ou privados, incluíndo a horticultura, floricultura, silvicultura, fruticultura, pastos e cultivos comerciais de plantas ornamentais, aromáticas e medicinais. Algúns caracois terrestres poden alcanzar o status de pragas mesmo en rexións relativamente áridas, pero as lesmas son especialmente problemáticas en climas tépedos e chuviosos. Varias especies de lesmas do xénero *Deroceras* dispersáronse por case todo o mundo grazas ao home, e isto é particularmente certo no caso de *Deroceras reticulatum* (Müller, 1774), unha exitosa especie oportunista e invasora, considerada como a babosa causante de pragas máis prexudicial, a nivel mundial. Actualmente *D. reticulatum* atópase na maioría das rexións tépedas e subtropicais, incluíndo Europa, Asia, Australia, Nova Zelandia, Tasmania, Canadá, Estados Unidos, Sudamérica, Sudáfrica e moitas illas dos océanos Pacífico e Atlántico.

O método máis común para o control das lesmas é o uso de molusquicidas químicos, que xeralmente son pellets ou cebos que conteñen como ingrediente activo entre un 2% e un 8% de metaldehído ou de metiocarbamato. Ambos compostos molusquicidas teñen impactos negativos sobre outros organismos, como escaravellos da familia Carabidae, que á súa vez son importantes depredadores de gasterópodos terrestres no campo, e tamén sobre animais domésticos e silvestres, especialmente aves e mamíferos. De forma relativamente recente comezaron a comercializarse cebos molusquicidas que conteñen fosfato de ferro como ingrediente activo, e que reivindican ser produtos totalmente naturais e seguros para a fauna e os animais domésticos. Nembargante, investigacións recentes demostraron que os molusquicidas con fosfato de ferro teñen tamén efectos adversos sobre a fauna; en particular demostrouse que afectan á supervivencia, crecemento e actividade de lombrigas terrestres, e existen indicios de que tamén representan un perigo para outros invertebrados do chan, así como para animais domésticos e para humanos, especialmente os cativos.

Respecto aos inimigos naturais, as lesmas e os caracois son depredados por unha ampla gama de vertebrados e invertebrados, e son parasitados por numerosas especies de dípteros, nematodos e ácaros. No caso da maioría destes inimigos naturais, é pouco probable que poidan realizar un control efectivo no campo das poboacións de gasterópodos prexudiciais, pero numerosas investigacións demostraron que os carábidos e os nematodos presentan un elevado potencial como axentes de control biolóxico de gasterópodos. De feito, o único axente de control biolóxico de gasterópodos que se comercializa é o nematodo *Phasmarhabditis hermaphrodita* (Schneider, 1859).

Unha das consecuencias do cambio climático global é un aumento da expectativa de maiores problemas ocasionados polas pragas agrícolas, así como dun incremento no desenvolvemento de resistencia aos praguicidas por parte de moitas especies daniñas. Ante estas expectativas, o control biolóxico de pragas aparece non só como unha opción non agresiva para o medio ambiente, senón tamén como unha alternativa con elevadas probabilidades de éxito para manter as poboacións de organismos causantes de pragas por baixo de límites aceptables, tanto no caso de pragas xa establecidas como no caso de potenciais novas especies invasoras. Desde este punto de vista, o control biolóxico de pragas convértese nun obxectivo prioritario de investigación, co fin de contribuír a adaptar a agricultura ás condicións climáticas cambiantes. Unha primeira fase, esencial, represéntana os estudos dedicados á identificación de inimigos naturais que poidan ser utilizados como axentes de control biolóxico de pragas. Os coñecementos actuais neste ámbito indican que os inimigos naturais xeneralistas, é dicir, aqueles capaces de atacar a unha ampla gama de presas, son os máis prometedores como axentes de control biolóxico de pragas nun ambiente cambiante.

É evidente que o cambio climático non só afecta as especies causantes de pragas, senón tamén aos seus inimigos naturais, á calidade e/ou cantidade de alimento dispoñible para elas, e a moitos outros factores. En función disto, as proxeccións realizadas polos modelos de predición das respostas ao cambio climático por parte de calquera organismo deben ter en conta, non só ás especies de interese en particular, senón tamén aos seus inimigos naturais e as súas relacións ecolóxicas. O éxito dos modelos depende en gran medida dos parámetros que utilicen e da súa validación por medio de evidencias experimentais, tales como manipulacións experimentais da temperatura e a precipitación, as cales xa teñen proporcionado valiosa información sobre as respostas de diferentes compoñentes dos ecosistemas terrestres ao cambio climático. Este é un paso previo fundamental para a modelización da resposta funcional dos agroecosistemas ao cambio climático, non só a nivel de especie senón a escalas máis amplas. Estudos previos de modelización suxiren que a abundancia, o rango de distribución, e a intensidade dos danos causados por *D. reticulatum* poden aumentar nos próximos anos en Europa debido aos efectos do cambio climático, pero devanditos modelos só tiveron en conta as propiedades fisiolóxicas e as relacionadas coa dinámica poboacional da propia especie.

O obxectivo principal deste traballo de doutoramento é explorar potenciais modificacións no comportamento alimentario da lesma *Deroceras reticulatum*, así como nos comportamentos dalgúns dos seus inimigos naturais máis importantes, baixo condicións climáticas pronosticadas para Galicia no último terzo do presente século. Os inimigos naturais estudados foron os escaravellos *Harpalus rufipes* (De Geer, 1774) e *Poecilus cupreus* (Linnaeus, 1758), e o nematodo zooparasito *Phasmarhabditis hermaphrodita*. Para alcanzar este obxectivo xeral realizáronse diferentes experimentos baixo condicións controladas no laboratorio, e no exterior baixo condicións semi-naturais.

No Capítulo 1 preséntanse dous experimentos deseñados para avaliar a resposta de *D. reticulatum* fronte a diferentes manipulacións climáticas, en termos de consumo de plantas cultivadas. Para iso cuantificouse o dano inflixido por poboacións de lesmas a plántulas de leituga. Estes experimentos realizáronse ao aire libre baixo condicións semi-naturais. As manipulacións climáticas que se utilizaron emulan condicións climáticas previstas para o Noroeste de España, en concreto un quecemento invernal e un aumento da precipitación no verán. No experimento de inverno comparouse un tratamento que representa un inverno normal, cun tratamento de quecemento invernal, caracterizado por temperaturas máis altas, menor humidade relativa e ausencia de choiva. No experimento de verán comparouse un tratamento que representa un verán normal sen choiva, cun tratamento de verán con precipitación, caracterizado pola presenza de choivas, mentres que as condicións de temperatura e humidade relativa foron similares en ambos os tratamentos de verán. Os resultados mostraron cambios significativos na cantidade de dano ocasionado ás plantas por *D. reticulatum* baixo as condicións climáticas previstas, o cal suxire un incremento do potencial desta lesma para causar danos aos cultivos no futuro.

No Capítulo 2 preséntase unha serie de experimentos de laboratorio deseñados para valorar a capacidade do escaravello *H. rufipes* para depredar sobre ovos e sobre babosas de diferentes tamaños, da especie *D. reticulatum*, tanto en ausencia como en presenza de presas alternativas. É ben coñecido que moitas especies de escaravellos polífagos son importantes depredadores de gasterópodos, pero a maioría dos estudos realizados ata a actualidade centráronse en só unhas poucas especies de escaravellos. *H. rufipes*, nembargante, recibiu pouca atención, aínda cando é un dos escaravellos máis comúns e abundantes en todo tipo de agroecosistemas na maioría dos países europeos. Os resultados demostraron que *H. rufipes* é capaz de matar considerables cantidades de ovos e de pequenos xuvenís de *D. reticulatum*, tanto en situacións nas que non existe alternativa como en situacións con outras presas disponibles. Isto suxire que *H. rufipes* podería realizar unha importante contribución ao control das poboacións de *D. reticulatum*.

No Capítulo 3 descríbense dous experimentos, un realizado baixo condicións controladas de laboratorio, e outras baixo condicións semi-naturais, deseñados para estudar o efecto da temperatura sobre a actividade depredadora dos escaravellos *H. rufipes* e *P. cupreus* sobre os ovos de *D. reticulatum*. No experimento de laboratorio utilizáronse cinco temperaturas constantes diferentes, e no experimento ao aire libre comparouse a actual temperatura ambiente de primavera-verán, cunha temperatura manipulada experimentalmente para emular as condicións previstas de quecemento. En ambos os experimentos *H. rufipes* destruíu máis ovos que *P. cupreus* e tamén foi a única especie cuxa actividade depredadora aumentou de xeito significativo co aumento da temperatura, o que suxire que o control biolóxico realizado por *H. rufipes* sobre *D. reticulatum* pode verse favorecido baixo as condicións previstas de quecemento.

No Capítulo 4 compárase a eficacia como axente de control biolóxico de *D. reticulatum* do nematodo *P. hermaphrodita*, baixo condicións invernais actuais e baixo condicións de quecemento invernal. En presenza do nematodo, tanto o dano causado polas lesmas ás plantas de leituga, como a supervivencia das lesmas, foron menores baixo as condicións de quecemento invernal. En ausencia de nematodos, o dano causado polas lesmas e a súa supervivencia foron similares en ambas as condicións. Polo tanto, os resultados suxiren que a eficacia de *P. hermaphrodita* como axente de control biolóxico de *D. reticulatum* pode aumentar nas condicións previstas de quecemento.

En definitiva, os resultados obtidos indican que tanto a lesma *D. reticulatum*, como os seus inimigos naturais *H. rufipes* e *P. hermaphrodita*, veranse afectados de xeito significativo polas condicións climáticas previstas. En concreto, os resultados sinalan que será de esperar unha maior capacidade das poboacións de *D. reticulatum* para ocasionar danos aos cultivos, e unha maior eficacia de *H. rufipes* e *P. hermaphrodita* para actuar como axentes de control biolóxico desta especie de lesma. Estes achados salientan a importancia de ter en conta aos inimigos naturais e ás interaccións ecolóxicas entre especies á hora de modelizar a resposta das especies a futuras condicións climáticas.

Do presente traballo de investigación extraéronse as seguintes conclusións xerais:

1. As condicións climáticas previstas de quecemento invernal e de aumento das precipitacións no verán provocaron un aumento no dano causado pola actividade alimentaria de poboacións de *Deroceras reticulatum* ás plantas cultivadas. Dada a súa adaptabilidade a diversos ambientes e climas, así como a súa ampla distribución xeográfica, no futuro cabe esperar un aumento dos danos causados por esta especie de lesma á produción agrícola.

2. O escaravello polífago *Harpalus rufipes* é capaz de localizar e matar ovos e pequenos xuvenís de *D. reticulatum*, e a súa actividade depredadora sobre estas presas aumenta se aumenta a temperatura. Dado que *H. rufipes* é un dos escaravellos terrestres máis comúns e abundantes nos agroecosistemas europeos, e dado que responde ós incrementos de temperatura previstos para o futuro aumentando a súa abundancia e actividade, cabe esperar que *H. rufipes* desempeñe un importante papel como antagonista natural de *D. reticulatum* en condicións climáticas futuras.

3. Fronte á depredación por parte de escaravellos xeneralistas como *H. rufipes* e *Poecilus cupreus*, os ovos das lesmas son máis susceptibles que as lesmas mesmas, incluídos os xuvenís máis pequenos. Isto implica que as especies de escaravellos capaces de destruír os ovos das lesmas poden realizar unha contribución significativa ao control das poboacións causantes de pragas. Con todo, a existencia de depredación sobre os ovos das lesmas por parte dos seus inimigos naturais é difícil de pór de manifesto, de maneira que este tipo de depredación debe de ser obxecto de máis investigación.

4. Un incremento de aproximadamente 4.5 ˚C na temperatura media de inverno resultou nun aumento da eficacia da cepa comercial de *Phasmarhabditis hermaphrodita* como axente de control biolóxico de *D. reticulatum*. Dado que *P. hermaphrodita* é un parásito facultativo, capaz de vivir como saprobionte asociándose a unha ampla gama de bacterias, pero mantendo a súa virulencia para *D. reticulatum*, e dado que este nematodo é común e abundante nos chans dos agroecosistemas europeos, cabe esperar

que en zonas nas que o cambio climático provoque un aumento das temperaturas invernais de xeito que estas se aproximen aos 14 ˚C, se produza un aumento da eficacia de *P. hermaphrodita* como antagonista de *D. reticulatum*, e este aumento de eficacia debera ser aplicable tanto á cepa comercial como ás poboacións naturais de *P. hermaphrodita*.

5. Nas zonas de produción agrícola deberan implementarse, e avaliarse, medidas destinadas a favorecer o establecemento e a proliferación de poboacións de escaravellos xeneralistas e outros inimigos naturais, das babosas, e de moitas outras pragas, posto que unha das estratexias máis prometedoras para combater as pragas agrícolas no contexto do cambio climático, é fomentar e aproveitar ao máximo o servizo que proporcionan os inimigos naturais para o control de pragas.


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Unwanted pests have hindered human efforts in land management since the onset of organized agriculture in the Neolithic, so that pest management has become an integral aspect of human civilization. There is an urgent need to meet increasing food demands by increasing crop yields, and satisfy sustainability goals by reducing reliance on synthetic pesticides. This is a challenging task for twenty-first century agriculture, further complicated against the background of climate change. Improved biological control appears as one of the most promising strategies. The research presented here explores potential changes, under predicted climatic conditions, in the feeding behaviour of the pest slug *Deroceras reticulatum*, as well as in the behaviours of some of their most important natural enemies, namely the carabid beetles *Harpalus rufipes* and *Poecilus cupreus*, and the slug parasitic nematode *Phasmarhabditis hermaphrodita*.

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