LISTE OF ABREVIATIONS

EAFRD: European Agricultural Fund for Rural Development

FAO: Food and Agriculture Organization of the United Nations

FRAB: Fédération Régionale des Agrobiologistes de Bretagne

GMO: Genetically Modified Organism

IGP: Intraguild Predation

NCBI: National Center for Biotechnology Information

GLOSSARY

[1]Biological control: the use of living organisms or their products for preventing or reducing damages caused by pests to crops.

[2]Entomopathogen: a pathogen agent (viruses, bacteria, rickettsia, fungi, protozoans and nematodes) that can cause disease to insects. Their natural occurrence in invertebrate populations contributes to the regulation of injurious pests of crops, households and domestic animals.

[3]Holometabolous: (complete metamorphosis) a type of metamorphosis in which an insect goes through four distinct stages: egg, larva, nymph, imago.

[4]Indigenous: an organism that is naturally occurring in a specific area.

[5]Intraguild predation: a natural enemy feeding on another natural enemy when both share another resource as food (usually plant-feeding prey or host species). As such, the interaction may involve either predation (or parasitism) and competition.

[6]Natural enemy: living organism found in nature that kills, weakens, or reduces the reproductive potential of other organisms.

[7]Parasitoid: an organism that develops within the body of another organism (host), feeds from it and finally kills it as a direct or indirect result of its development.

[8]Scavenging: feeding behavior of some organisms (including some arthropods) in which the scavenger feeds on dead animal present in its habitat.

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1. INTRODUCTION

1.1. Background

French livestock farming is strongly dependent on the importation of protein rich raw materials, mainly South American soybean. Between 2011/12 and 2016/17 France imported 3.2 million metric tons of soybean meal per year on average (France: Agricultural Biotechnology Annual, 2017), which roughly represented half of the total cattle feed consumption in France (Peyronnet, 2014). 75% of this product is labeled as genetically engineered (mainly glyphosate-tolerant varieties) and Brazil is the leading supplier with a 70% market share in 2015/16 (France: Agricultural Biotechnology Annual, 2017). Yet, there is a strong social demand in France for GMO free feedstuffs (Labalette *et al*., 2010). This allows a better valorization for local protein sources (Peyronnet, 2014). Reducing French dependence on imported protein rich raw materials is also an important goal for French agricultural and livestock farming competitiveness, especially when the global increase of needs (e.g. in China) makes the market of soybean under pressure. French deficit on this kind of product has already been reduced from 70% in the 1980's, to less than 40% in 2010 (Peyronnet, 2014). France (and Europe) continues working on its way into protein self-sufficiency through the evaluation of potential sustainable soy cake alternatives, like the development of local protein crops based on different leguminous, such as pea (*Pisum sativum* L.), lupine (*Lupinus albus* L.) or our subject of study, faba bean (*Vicia faba* L.) (fig. 1).

In the Great West region of France (Bretagne and Pays de la Loire), the research and experimentation program SOS PROTEIN (Sustain Our Self-sufficiency Protein Research to Overcome the Trend of European Import Needs), coordinated by the "Pôle Agronomique Ouest", seeks to address this issue. SOS PROTEIN is divided in four main axes: 4AGEPROD (FORAGE PRODuction), DY+ (Digestibility increase), TERUnic (Territory Economics the Right Understanding), and the PROGRAILIVE project (Production PROtein GRAIn for LIVEstock), to which the present study contributes. Funded by the European Agricultural Fund for Rural Development (EAFRD*) and the Regions Bretagne and Pays de la Loire, PROGRAILIVE aims to secure and increase the production of leguminous grains for breeding in Western France. Among possible factors that put yields at risk, several insect pests (aphids, weevils and bruchids) may cause significant damages to the crops although their exact contributions remain largely unknown. As part of an agro-ecological approach to pest management, the project seeks to better understand the natural pest control provided by natural enemies[6] on pea, lupine and faba bean, experimenting on farmsteads using mixed crops (cereal and legume intercropping). One of the main hypotheses tested is that mixed crops may improve the ecosystem service of biological control[1] compared with pure stands.

Figure 1: Faba bean plant, *Vicia faba* L. A. stalk with flowers; B-D. flowers; E. dry seeds; F. plant; G. stalk with pods; H. pods with seeds; I. fresh seeds
Source: José Vicente Santamaría Monsoríu

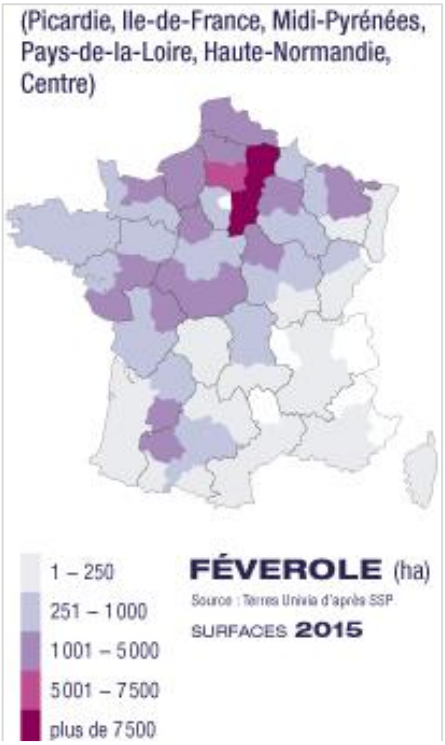

Figure 2: Main production areas of faba bean in France in 2015 based on surface. Source : Terres Univia from SSP. 2015)

Figure 3: *Sitona lineatus* (L.) adult. Source: U.Schmidt, 2013

Figure 4: *Sitona lineatus* (L.) larvae feeding from root nodules of a leguminous plant.
Source: Carré S. (INRA)

Figure 5: *Sitona lineatus* adults feeding from a leguminous plant leaf.
Source: www.pflanzenkrankheiten.ch/

Grain legumes constitute a source of N incorporation into the soil via N2 fixation (Corre-Hellou and Crozat, 2005). They play a key role in reducing external N inputs, notably in organic cropping systems. This N2 fixation is possible thanks to the root nodules that leguminous plants form in symbiosis with bacterial species of the genus Rhizobium. These bacteria synthesize an enzyme, nitrogenase, essential for N2 fixation. Reciprocally, plant provides to bacteria an intracellular environment favorable to its development, as it is rich in the carbohydrates they need to grow. Besides being a cash crop, faba bean (also broad bean, field bean or horse bean), *Vicia faba* L. (Family Fabaceae) (fig. 1), has been cultivated from early Neolithic times, beginning in the Near East and spreading since then all over the world (Duc, 1997). Faba bean remains nowadays an important winter crop in warm temperate and subtropical areas (Duc, 1997), mainly for forage production as it constitutes a major protein source for cattle feed. In France, the main producer regions in 2015 were Picardie, Ile-de-France, Midi-Pyrénées, Pays-de-la-Loire, Haute-Normandie and Centre (Terres Univia, 2018) (fig. 2). In biological agriculture, faba bean is frecuently conducted as an intercrop with cereals (40% of the surfaces in Biological Agriculture in Bretagne Region, France. Observatoire de la production bio 2015; FRAB*) as this practice often leads to various agronomic, environmental and economic benefits such as the reduction in fertilization needs, pest and weed control benefits (Corre-Hellou and Crozat, 2005) or the increasing of yield stability (Raseduzzaman, 2016).

1.2. The pea leaf weevil, a significant pest of legume crops

Among main pests of faba bean in France may be found several fungal pathogens like *Ascochyta fabae* or Botrytis fabae, nematodes as *Ditylenchus dipsaci* and viruses as BLRV (Bean leaf roll virus) and BYMV (Bean Yellow Mosaic Virus). The broomrape species of parasitic plant *Orobranche crenata* is also a common problem for faba bean crops in France (ARVALIS - Institut du vegetal, 2013). Concerning arthropods, faba bean faces over 70 species of pests all over the world (Stoddard *et al*., 2010). Several common insect pests may cause significant damages to faba bean. The black bean aphid *Aphis Fabae* (Hemiptera: Aphididae) forms dense colonies affecting plant growth and flowering due to the action of toxic saliva and vectoring plant viruses. The bruchid species *Bruchus rufimanus* (Coleopera: Bruchidae) is another worldwide pest of faba bean. The complete larval development takes place inside the seed, reducing yield mass, quality and affecting germinative properties. And finally, *Sitona lineatus* L. (Coleoptera: Curculionidae) (fig. 3), commonly known as the pea leaf weevil, the species constituting the focus of this study.

S. lineatus is a significant pest of leguminous plants native to Europe and North Africa which has been introduced into many countries all around the world (Olfert *et al*., 2012). Its geographical range and abundance expand in association with the extension of host plants crops (Landon *et al*., 1995), current agricultural practices and climate change (Vankosky *et al*., 2010; Olfert *et al*., 2012). The pea

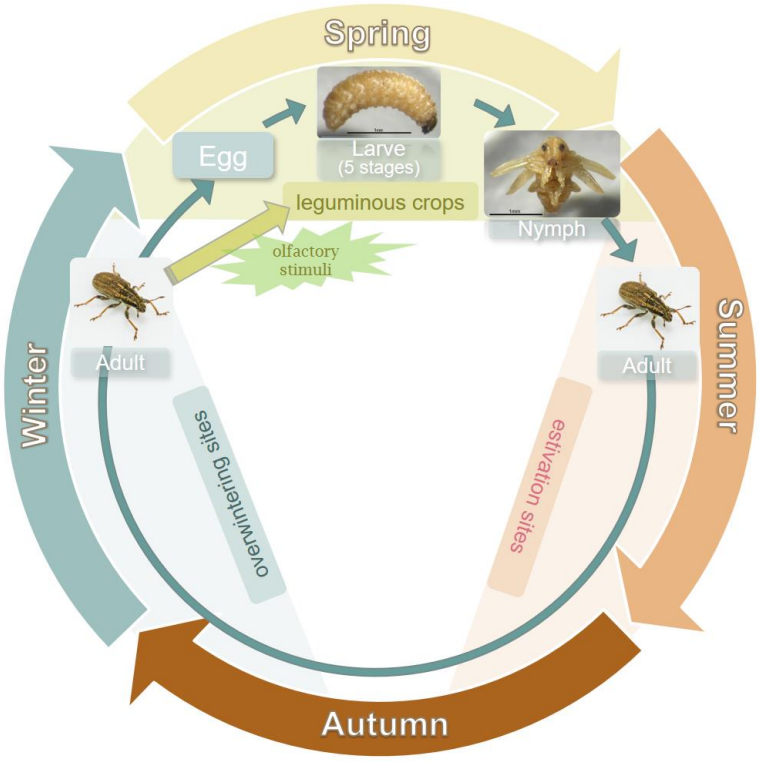

Figure 6: Biological Life Cycle of *Sitona lineatus* (L.)
Source: María Romá-Mateo

Figure 7: left, *Sitona lineatus* (L.) adult; right, typical U-shaped notches on leaves caused by *Sitona lineatus* (L.) adults when feeding.
Source: Antonio Robledo ©

leaf weevil main host plants are pea (*Pisum sativum* L.), faba bean (*Vicia faba* L.), lucerne (*Medicago sativa* L.), medick (*Medicago lupulina* L.), all species of clover (*Trifolium* sp.), and wild vetches (*Vicia sativa* L.) (Jackson, 1920). Post-diapause adults prefer field pea and broad bean (Landon, 1995). Nevertheless, some other species of perennial legumes can serve as secondary hosts during the periods when annual legumes are absent (Vankosky *et al*., 2010).

Yield losses caused by this pest result mainly from larval damage when feeding from root nodules (fig. 4), and not from adult feeding on leaves (fig. 5) (Cantot, 1989; Doré and Meynard, 1995; Vankosky *et al.*, 2011). It has been estimated that yield reductions can reduce production in a 5-10%, reaching 10 quintals per hectare in some cases, as root nodules destruction can result in partial or complete inhibition of nitrogen fixation (Landon, 1995). As a result, the cropped plant may turn to partially heterotrophic for nitrogen, thus negating the expected benefits from fixing atmospheric nitrogen in the soil. This process is not directly observable and hardly quantified. Hence, the effects of the pea leaf weevil on nitrogen grain content and soil nitrogen balances are probably overlooked by farmers cultivating leguminous crops.

The life cycle of *S. lineatus* is holometabolous[3], comprising an egg stage, 5 larval instars and a pupal stage preceding the adult stage (fig. 6). Under French conditions, the pea leaf weevil produces one generation per year (Cantot and Rolet, 1986). In March/April, adults migrate from overwintering sites to annual legume crops (Cantot and Rolet, 1986) which they locate likely via olfactory stimuli originating from hosts plants, as suggested by Hardwick and Harens (2000) after a choice test experiment with *Sitona lepidus* Gyllenhal, a species of weevil closely related to *S. lineatus*. The exact date of arrival to leguminous crops varies according to the region (Jackson, 1920) and climate conditions. Once there, they start feeding immediately out of the host plants leaves, causing characteristic U-shaped notches (Cantot and Rolet, 1986) (fig. 7) which are, consequently, a typical evidence of the first appearance of the weevil (Jackson, 1920). Since *S. lineatus* is present on a host plant crop, adult males are able to produce an aggregation pheromone, 4-methyl-3,5-heptanedione, attracting likewise males and females as they are both capable of perceiving it (Blight and Wadhams, 1987). Soon after males and females converge, they start reproducing and mated females lay their eggs essentially on the soil surface, but they can also lay on the plant leaves (Cantot and Rolet, 1986). *S. lineatus* has high fecundity rates; an adult female deposits more than 1000 eggs during its whole adult life (Schotzko *et al*., 1986). Eggs take around 3 weeks to hatch (Jackson, 1920) and neonate larvae immediately dig into the soil surface in search of the legume root nodules they feed on (Cantot and Rolet, 1986). Around 6-7 weeks after hatching, pupation takes place (Jackson, 1920) and the immobile pupal stage develops underground (Cantot and Rolet, 1986). In late June/early July a new generation of emerged adults migrates into their estivation sites where they feed until October to finally reach the overwintering sites (Cantot, 2001).

1.3. Agroecological pest management

Agricultural systems face with the major challenges of climate change, human demography, sustainability and health. This advocates a paradigm shift away from conventional intensive systems to a new model for agricultural production. Innovative cropping systems must stay productive and with more socio-economic benefits, but natural resources must be preserved and healthy ecosystems and human well-being must be guaranteed.

Agroecology is defined by the FAO* as the scientific discipline that studies how different components of the agroecosystem interact. According to this organisation, agroecology can support food production as well as food security and nutrition while restoring the ecosystem services and biodiversity that are essential for sustainable agriculture. Therefore, agroecology emphasizes on the incorporation of ecological principles into pest management and, at the same time, ensures high productivity and profitable harvests without causing harm to the environment (Wang, 2014).

In order to design sustainable pest management strategies for the pea leaf weevil, a deep understanding of the species ecology and its interactions with its natural enemies is needed. Thereafter, efficient agroecological methods of crop protection should be developed via conservation biological control (Vankosky *et al*., 2010).

Conservation biological control is the implementation of practices that enhance the local abundance, reproduction and survival of natural enemies[5] of pests (McCravy, 2008). Thus, conservation biological control programs in agroecosystems aim to improve natural pest control by providing indigenous[4] natural enemies with high quality resources, whether they be complementary or supplementary resources. Resources range from the field and its vicinity, offering shelter (e.g. field margin vegetation), alternative preys or hosts and sugar sources (flowers) to landscape with suitable habitats and corridors between populated patches (Dent, 1995; Eilenberg *et al*., 2001, Pollier *et al*. 2018). Some agricultural practices can favor natural enemy conservation, like the reduction of pesticide use, selective insecticides, low tillage, long crop rotation, winter seeding or intercropping (Hanavan, 2008; Vankosky *et al*., 2011). No till systems and organic production are, of course, good candidates for implementing conservation biological control. Indeed, intercropping appears to have strong potential for controlling the impact of many legume pathogens and pests (Vankosky *et al*., 2010; Corre-Hellou *et al*., 2011), as well as for significantly increasing natural enemies populations (Wiech, 1991). For instance, a study about the biological control of lepidopteran stem-borers in cereal crops in Africa showed that intercropping with non-host species can, not only directly reduced pest infestation levels by releasing volatile substances repealing female stem-borers,

but also increased parasitism levels by a natural enemy that is attracted by one of these substances (Kahn, 2000). When including intercropping for the management of a particular pathogen or pest, a case by case approach must be undertaken, but as a general rule the presence of a second (or more) species in the crop can entail a negative impact on the attacker species population in one of the three following ways: directly interfering with the attacker, changing the original crop attractiveness, or favoring natural enemies populations (Trenbath, 1993). From this premise, our study takes this agricultural practice into account for the evaluation of *S. lineatus* biogical control.

Potential indigenous predators of *S. lineatus* have already been studied, including staphylinid and carabid beetles by estimating predation rates in the laboratory through exposure egg predation tests in Petri dishes (Vankosky *et al*., 2010), or adult and egg predation in the field by radio- labelling experiments (Hamon *et al*., 1990). Also egg and adult hymenopteran parasitoids[7] can cause significant *Sitona* sp. mortality rates at high host densities in the field (Aeschliman, 1980). Entomopathogenic[2] fungi, like *Metarhizium flavoviride*, can also efficiently attack young stages (eggs and neonate larvae) of *S. lineatus* (Poprawski *et al*., 1985), and *Metarhizium anisopliae* has been tested effective against pea leaf weevil larvae, pupae and adults (Verkleij *et al*., 1992). However, most of this research was based on experimental set up in the lab or complex and highly time-consuming field studies. Laboratory predation experiments can provide useful information on potential predators but neither demonstrates real predation in the field nor estimates field predation rates.

1.4. PCR molecular gut-content analysis

It has been over six decades now since biochemical techniques were first used to detect arthropod prey-predator relationships in the fields from predators' stomach content analysis (Boreham and Ohiagu, 1978). Most efficient traditional methods were based on the development of specific monoclonal antibodies against the prey (Boreham and Ohiagu, 1978; Sunderland, 1988; Greenstone 1996). Serological techniques, despite being satisfactorily sensitive and specific, remain long, complex and expensive (Greenstone *et al*., 1996). These days it is widely accepted that molecular PCR-based techniques for tracking predator-prey interactions in field studies constitute a versatile, reliable and efficient method (Agustí *et al*., 1999; Zaidi *et al*., 1999; Chen *et al*., 2000; Symondson, 2002; Harper *et al*., 2005).

When targeting a single species of prey, a PCR can be performed using a specific primer against the target prey DNA. This technique is nowadays increasingly being used in agroecological studies for identifying trophic links between insect pests and their natural enemies (Wolf *et al*., 2018; Albertini *et al*., 2018; Peterson *et al*., 2018), the final goal being to unravel the trophic basis from which future efficient conservation biological control strategies will be developed. For *S. lineatus*, only Harper, King

Figure 8: Pitfall trap used for predator captures in faba bean plots. Source: María Romá-Mateo

et al. (2005) carried out gut-content molecular analysis for its detection into predators' guts. They developed a multiplex PCR-based rapid screening system capable of being used for the simultaneous detection of a large number of preys (including the pea leaf weevil). As a result of their research, they successfully found, among others, *S. lineatus* DNA in 16% of the tested predators, all belonging to the generalist carabid species *Pterostichus melanarius*.

1.5. Objectives

The first objective of the present study was to identify indigenous predators of *S. lineatus* in faba bean fields of Western France. To do so, PCR-based detection of *Sitona lineatus* DNA was carried out on predators caught in the fields. The contribution of each predator species to the pest biological control may vary according to several factors affecting directly or indirectly (through competition, intraguild predation[5] or resource availability for example) their abundances. We tested the effects of three factors on predator community structure and pea leaf weevil predation: first the seasonality, some species being precocious while others are late, second the crop type, distinguishing faba beans intercropped with cereals from pure stands, and third the location in the fields, comparing the periphery exposed to border effects with the field center. The factors effects were measured by comparing both the PCR detection results and the predator diversity and abundances. Disentangling the trophic relationships, their dynamics and determining factors provides the necessary knowledge to an agroecological engineering approach of pea leaf weevil management in leguminous crops.

2. MATERIALS AND METHODS

2.1. Plot network

The study sites were located in the Maine-et-Loire department of Western France, close to the cities of Savennières and Bouchemaine. The landscape was dominated by cereals, oilseed rape, vegetable fields and permanent grassland. From March to May 2018, 14 commercial fields were monitored and used for the sampling of the laboratory tests specimens. Half of the plots were conducted as bean monocropping fields, the other seven as cereal/bean intercropping fields. All of them had been grown in organic farming, except for two monocropped fields in Savennières.

2.2. Predator captures

For monitoring ground-active arthropod biodiversity and abundance, we conducted a mass-collection method using wet pitfall traps of 8 cm in diameter and 25 cm in depth (fig. 8) leveled with the ground surface. Each trap was filled with a saline solution as a killing preservative. Eight traps were

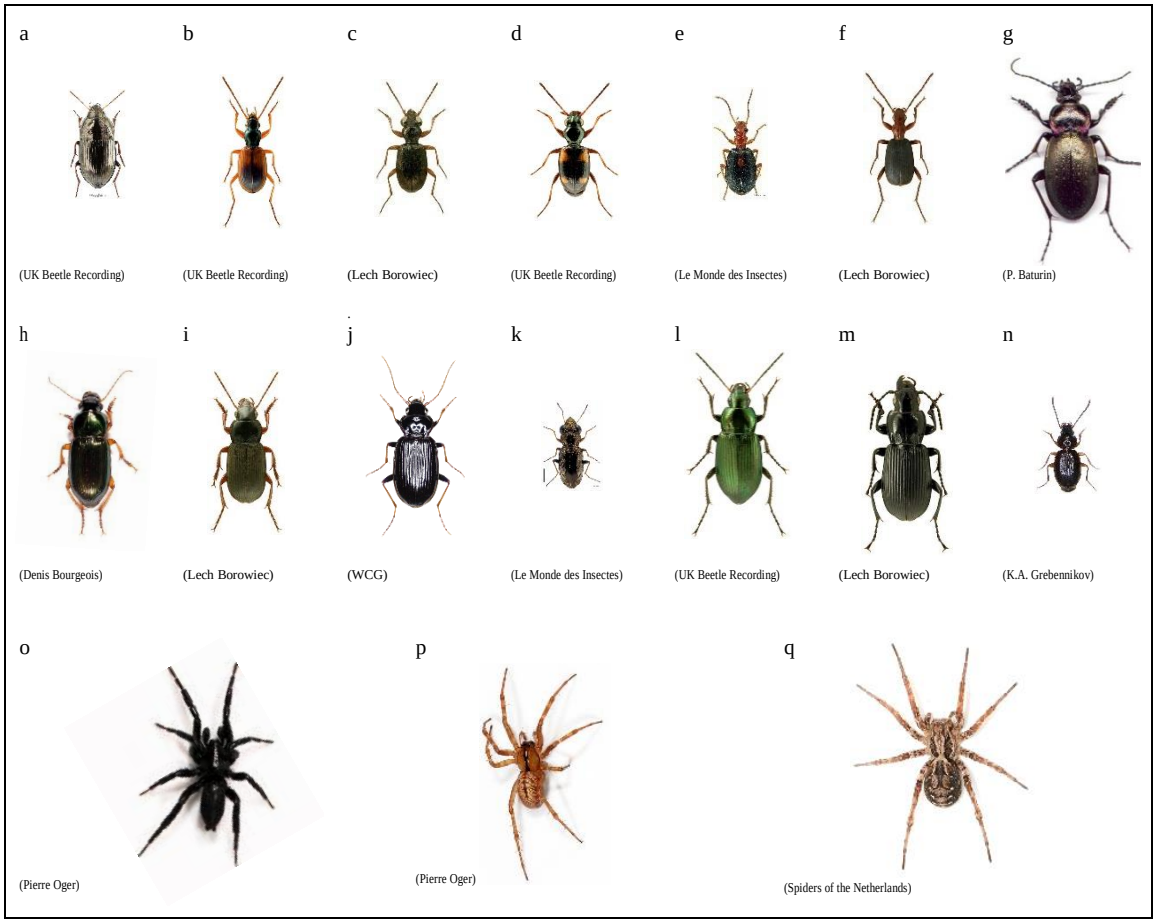

Figure 9: 14 groups of carabid beetles and 3 groups of spiders screened by PCR targeting *Sitona lineatus* (L.) DNA. a. *Amara* sp. b. *Anchomenus dorsalis*; c. *Asaphidion* sp. d. *Bembidion* sp.; e. *Brachinus sclopeta*; f. *Brachinus* sp.; g. *Carabus nemoralis*; h. *Harpalus affinis*; i. *Harpalus* sp.; j. *Nebria salina*; k. *Nothiophilus* sp.; l. *Poecilus cupreus*; m. *Pterostichus melanarius*; n. *Trechus* sp.; o. Family Gnaphosidae; p. Family Linyphiidae; q. Family Lycosidae

placed in each one of the plots along two linear transects of 4 traps each with a minimum of 10 m trap separation (Firlej *et al*. 2013). The first transect was placed in the center (i.e. >30m from the field edge) of each plot; the second was installed lengthwise of the plot margin, at 3-4m from the field border. Before each sampling, traps remained open for one week. We realized one sampling by month from March to May.

For the molecular analysis, individuals were captured using the same pitfall trap system as described above, but without any solution added to the traps. Dry traps remained open for 24 h before sampling. After collection and during transportation, all individuals were kept on ice to prevent DNA degradation. Once in the laboratory, they were immediately killed by freezing and stored at -24°C (Wolf *et al*., 2018). We realized two samplings in March, three in April and one in May, that being six samplings in all.

2.3. Procedure for biodiversity and abundances analysis

From the large amount of arthropods collected for biodiversity and abundances assessments, we only selected those belonging to taxonomic groups having predator habits, according to our purpose. These groups are: carabid beetles (Coleoptera: Carabidae), rove beetles (Coleoptera: Staphylinidae), spiders, centipedes (Class Chilopoda), earwigs (Dermaptera: Forficulidae), harvestmen (Opiliones), and ladybugs (Coleoptera: Coccinellidae). Carabid beetles, earwigs and ladybugs were identified at the species level in most cases, otherwise, at the genus level. Rove beetles, spiders were identified at the family level, harvestmen at order level and finally centipedes at class level. All identifications were attained by visual morphological examination.

2.4. Sample preparation for molecular analysis

Prior to identification, the bench and instruments were cleaned with bleach and all individuals were bleach-washed in order to remove potential contamination on predator body surface (Greenstone *et al*. 2012; Wallinger *et al*. 2013). The decontamination method used, developed by Wallinger *et al*. (2013) consisted in bathing the specimens in 1-1.5% bleach solution for 30 s and then rinsing them twice with molecular-grade water. Individuals were then identified, placed individually in a 96-well plate and stored again at -24°C as quickly as possible waiting for DNA extraction.

22 groups of predator arthropods were selected for PCR analysis, including 14 species and genus of carabid beetles (fig. 9) and also rove beetles, harvestmen, centipedes, earwigs, ladybugs and three families of spiders (Gnaphosidae, Lycosidae and Linyphiidae) (fig. 9). For molecular analysis each group was identified in the same way and to an identification level identical as for biodiversity and abundances (described above).

In most cases, whole predators were crushed within the cluster tubes. Only *Carabus nemoralis* and *Poecilus cupreus* were dissected before analysis, since they were the biggest in size and some of their hardest body parts could have difficulted the DNA extraction process. For *P. cupreus* elytra and legs were cut off. For *C. nemoralis* the foregut was removed and only this part was analyzed.

At the moment of predator collection, the presence of *Sitona lineatus* in the field was verified indirectly by counting the number of pea leaf weevil attacks on faba bean leaflets. We randomly selected twenty plants per plot (ten in the vicinity of the first trap installed in the edge, ten in the vicinity of the first trap in the main crop) and counted all U-shape notches on the two top leaves of each plant. Crop growth and development was also recorded. Plant height and leaf number were measured on the same plants.

2.5. Predator gut-content molecular analysis

558 samples were screened targeting *Sitona lineatus* DNA for amplification. At the end of the present study (end of july 2018) we expect to achieve a number of screened predator samples of around 1800.

The primer pair sequences used to amplify the cytochrome oxidase subunit 1 (COI) gene from *Sitona lineatus* DNA were F1 (5′-AGCAAATATCGCACATGAAGG-3′) and R1 (3'-AAGAGGTGTCCGATCAAAGG-5') (Harper, King *et al*. 2005), and the PCR product length was 151 pb. The nucleotide sequences data appear in the NCBI* nucleotide sequence databases under Accession no. AJ865012. The sequence had been developed by Harper, King *et al*. (2005) in a study reporting for the first time a multiplex-system approach to detect several prey species simultaneously in the gut-content of invertebrate predators. They sequenced and characterized this pair of primers from a weevil of the genus Sitona, but they ignored which precise species it was (William Symondson, personal communication). Thus, before starting laboratory experiments, the specificity of the primer pair against *Sitona lineatus* was tested *in silico* through Blastn against the nucleotide collection database of NCBI* (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Prior to sample routine analyses, extraction and amplification protocols were both refined and tested with the aim of making sure that all conditions were fully optimised to detect *Sitona lineatus* DNA in a species-specific way within screened predators. For the optimisation of PCR conditions, a gradient test was performed in singleplex in order to identify the optimal hybridization temperature for the pair of primers against *S. lineatus* DNA. We also accomplished a test using different primer concentrations to find the optimal one. Finally, we tested the specifity against *Sitona lineatus* by PCR screening of different type of samples: 1 individual of a species taxonomically distant from *S. lineatus*, 5 different

individuals of starved carabid beetles, 1 *S. lineatus* individual, 1 *S. lineatus* egg, 3 *S. lineatus* eggs, 5 *S. lineatus* eggs and 2 carabid beetle individuals who had consumed *S. lineatus* eggs in an exposure experience in laboratory.

The total DNA of each sample was extracted using the commercial kit DNeasy® 96 Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany), following the manufacturer's instructions except for the following modifications: 1. First of all, we added two grinding balls into every well of the 96-well plate containing the samples; 2. Then we submerged the tubes containing the samples in liquid nitrogen and predators were crushed and homogenized right after with MM 301 mixer mills (Retsch GmbH, Haan, Germany) at 20 Hz for 30 s.; 3. Plates were then centrifuged to collect any material from the caps, which were afterwards replaced for new ones in order to prevent any contamination; 4. In the final step (elution), extracted DNA was recovered in a final volume of 30 µL elution buffer. Total extracted DNA was quantified with NanoDrop (Thermo Scientific NanoDrop Products), diluted 1/100 and stored at -20° until further analysis.

PCR were carried out in a Bio-Rad MyCycler Thermal Cycler PCR (Bio-Rad Laboratories, Hercules, California, USA) using the following cycling conditions: 95°C for 4 min followed by 40 cycles of 95°C for 30 s, 57°C for 90 s, 72°C for 90 s, and a final cycle of 72°C for 10 min. 1 µL of each sample DNA was amplified in 19 µL GoTaq® Master Mix (Promega, Madison, WI, USA), containing 0.25 µM each primer, 4 µL Taq polymerase buffer, 1.2 µL MgCl2, 0.5 dNTPs, 0.05 Taq polymerase, 12.75 µL ultrapure water. In order to ensure that results were not biased by PCR failure we also run a positive control containing *Sitona lineatus* tissu (one whole individual). Two different types of negative controls were included as well: one ultrapure water control and one springtail individual (Subclass Collembola).

PCR products were run in 2% agarose gel electrophoresis, which provided with binary data results, showing presence or absence of our target, *S. lineatus* DNA, in each predator sample.

3. RESULTS

3.1. Crop growth and pest damages on faba bean fields

The beginning of spring was very rainy, saturating the soil where roots were not able to grow normally. This was especially true in the center of the fields where faba bean plants were consistently smaller than near the field border, with an average height of 81.2±15.9cm (sd) and 94.3±16.6cm in mid-May, respectively. This growth gap did not affect the development of faba beans. Indeed, the number of counted leaves grew similarly in the two crop types and the two spatial plots throughout the season, from 5.2±1.1 leaves on March 29 to 18.7±2.8 on May 14. The phenological stages (flowering start, last flower

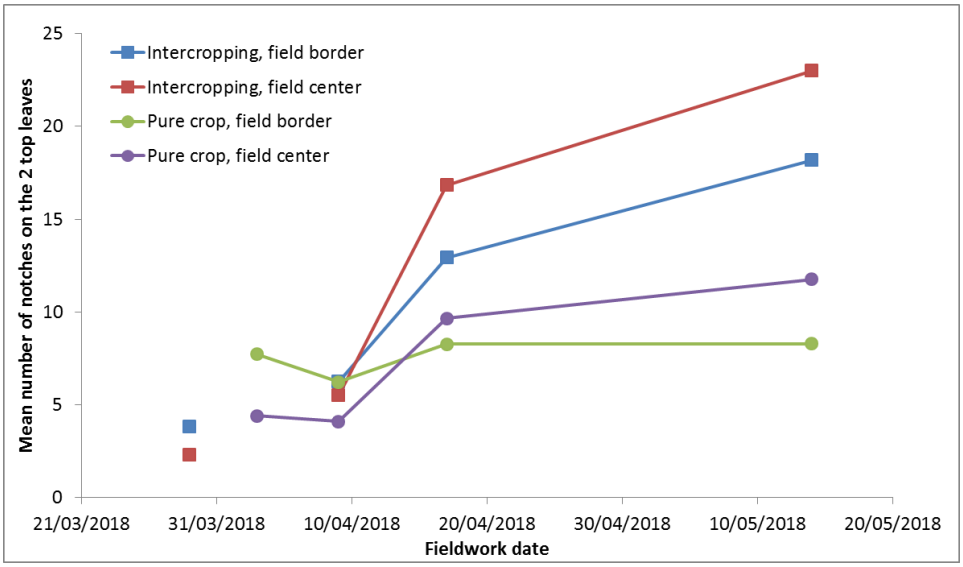

Figure 10: Assessment of the magnitude of pea leaf weevil (*S. lineatus* L.) damages on faba bean plants from the end of March to mid-May 2018. Damage calculated as the number of U-shaped notches on the two top leaves of the plant. 10 sampled plants by area of the plot (edge or main crop) and by plot.

Table 1: Biodiversity and cumulative abundances of predator arthropods caught from March to May 2018 in faba bean fields (preliminary data).

Group	Furthest level of identification	n
Carabid beetles	*Amara* sp.	2
	Anchomenus dorsalis	87
	Asaphidion sp.	1
	Bembidion sp.	3
	Brachinus sclopeta	54
	Brachinus sp. (other than sclopeta)	8
	Carabus nemoralis	9
	Harpalus affinis	13
	Harpalus sp. (other than affinis)	14
	Nebria salina	48
	Nothiophilus sp.	18
	Poecilus cupreus	21
	Pterostichus melanarius	1
	Trechus sp.	26
	Other carabid	83
Spiders	Family Gnaphosidae	15
	Family Linyphiidae	34
	Family Lycosidae	67
	Family Salticidae	1
	Family Thomisidae	1
Rove beetles		61
Harvestmen		11
Centipedes		8
Earwigs	*Forficula auricularia*	10
Ladybugs	Family Coccinellidae	1

and first pod>2cm) were also observed synchronously. The flood hampered cereal growth in intercropped fields where the cereal cover occasionally appeared sparse and heterogeneous.

Pest damages were only recorded by counting notches resulting from the feeding of *S. lineatus* adults. There were always much more notches in intercropped fields than in pure stands (fig. 10). At the start of the experiment, field colonization by adults from the field border resulted in more notches on top leaves for plants in the field periphery than for plants in the field center. This trend reversed during the season as the pea leaf weevil dispersed toward the interior of the fields (fig. 10).
Over the 1100 leaves observed during the season, less than 10% (109) were injury-free. This percentage dropped below 2% on May 14. Hence, pea leaf weevils were abundant and active on every spatial plot of the field network.

3.2. Predators biodiversity and abundances

The analysis of biodiversity and abundance was based on arthropods captured from March to May 2018. From the large amount of arthropods collected we have classified for now 1018 predators, distinguishing between them 580 carabid beetles, 347 spiders, 61 rove beetles, 11 harvestmen, 8 centipedes, 10 earwigs, and 1 ladybug (Table 1). A much greater amount of predators waits until identification and quantification for being included in further analysis.

Preliminary results in number of individuals collected show 7 predominant groups of carabid beetle species and three groups of spiders. For carabid beetles, the most abundant species is *Poecilus cupreus* (213 field-collected individuals), followed by *Anchomenus dorsalis* (87), *Brachinus sclopeta* (54), *Nebria salina* (47), the genus Harpalus (27), genus Trechus (26), and finally the genus Nothiophilus (18) (fig. 12). The most abundant families of the identified spiders so far were distributed in the following order: first spiders of the Family Lycosidae (67), then the Family Linyphiidae (34) and the Family Gnaphosidae (15).

The same taxonomic groups were found in both intercropping and monocropping plots, so the preliminary data show no effect of this agricultural practice on diversity.

For the analysis of the effect of our three factors of study (type of crop, zone of the plot and seasonality) on the groups found, we focused only in carabid beetles and in spiders of the 3 most abundant families. The rest of the groups cannot be consistently analyzed yet, as identification is still on process and for the moment we don’t have enough data about their abundances.

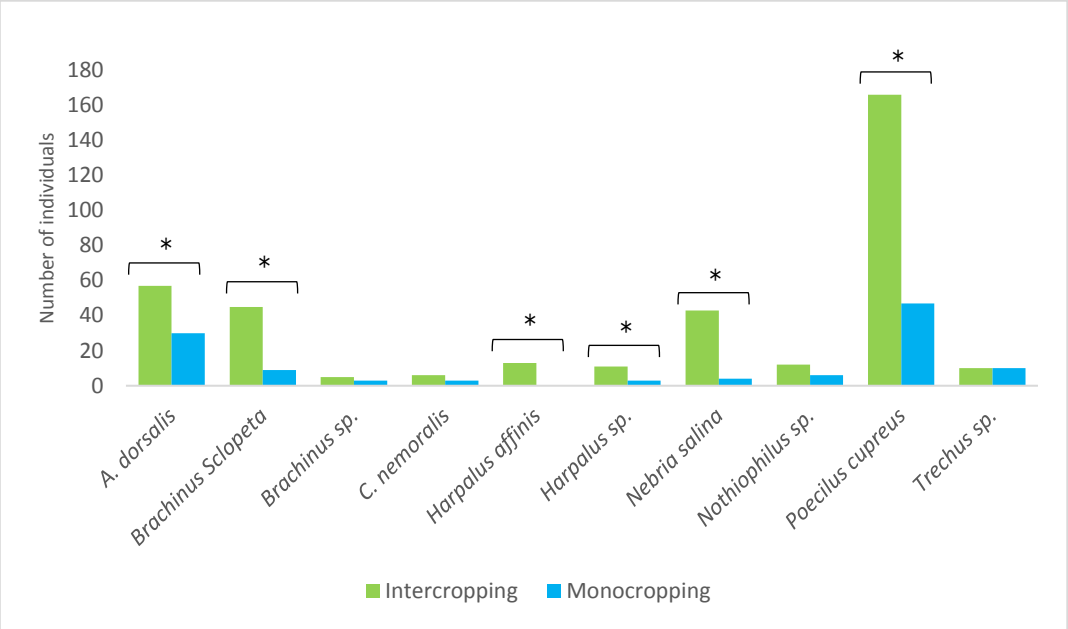

Figure 11: Cumulative abundances of carabid beetles caught from March to May 2018 in intercropped (n=7) and monocropped (n=7) fields of faba bean (preliminary data).

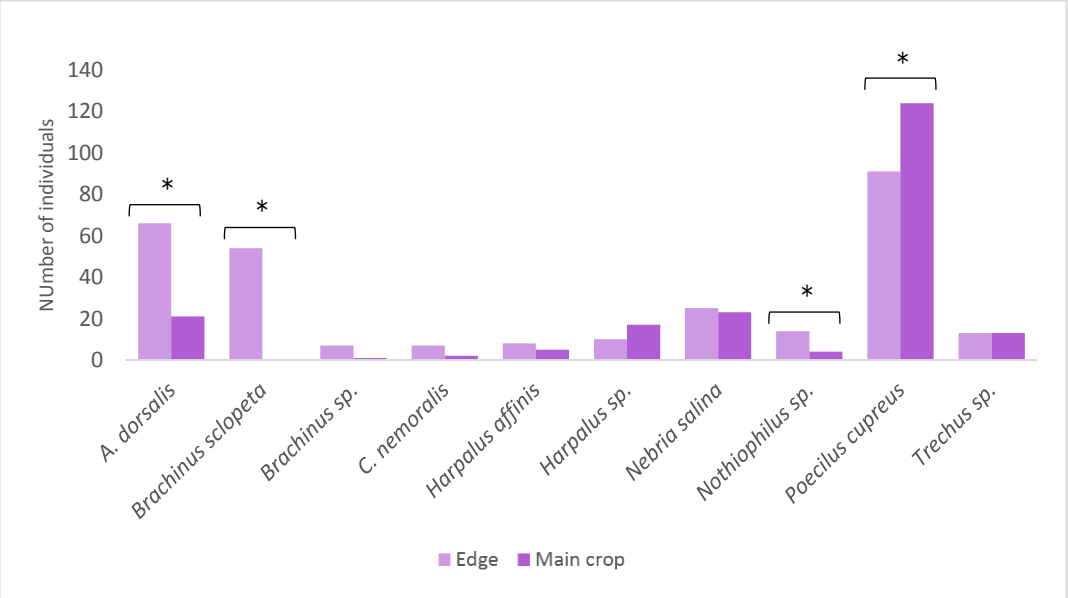

Figure 12: Cumulative abundances of carabid beetles caught from March to May 2018 in the crop edge and in the main crop of faba bean fields (n=14) (preliminary data).

3.2.a. Effect of intercropping on spider and carabid beetle abundances

For carabid beetles in general, many more individuals were caught in intercropping plots (n=425) than in pure stand ones (n=155) (χ^2 $p=3.6*10^{-29}$). This held true for the most abundant taxonomic groups with the exception of *Trechus sp.* (fig. 11). *Harpalus sp.* and *Nebria salina* were even caught on rare in the monocropped fields. This contrasts with spiders of the three most abundant families, found in similar numbers in intercrop and pure stand, with total abundances reaching 51 and 65 individuals, respectively.

3.2.b. Effect of plot area (edge or main crop) on spider and carabid beetle abundances

The total number of carabid beetles caught in the pitfall traps placed in the fields was significantly higher in the crop edge than in the main crop (χ^2 $p=3.29*10^{-5}$), with 340 and 240 individuals respectively. The activity-density of spiders was also greater near the field border than near the field center, with 78 and 38 individuals respectively. Specifically, 75% (n=67) of the spiders belonging to the *Lycosidae* family were trapped in the crop edge.

Comparing the carabid community in the 2 types of spatial plots (edge or main crop) revealed contrasted behaviors at the group level (fig. 12). Some groups were more abundant in the main crop (e.g. *Poecilus cupreus* (χ^2 p=0.0244)) while others showed marked preferences for the field edge (*Anchomenus dorsalis* (χ^2 $p=1.4*10^{-6}$), *Notiophilus sp.* (χ^2 p=0.0184)). The species *Brachinus sclopeta*, was always absent in the main crop but abundant in crop edge. For one taxonomic group, the *Trechus genus*, the distribution of catches was balanced between the 2 spatial plots.

3.2.c. Effect of seasonality on carabid beetle diversity and abundances

The impact of the different period of mass-collection captures was studied only for carabid beetles. As we realized a different number of captures each month (2 in March, 3 in April and 1 in May), we assessed the effect of seasonality on each species abundances comparing the proportion of individuals captured each month out of the total number of carabid individuals captured in that month.

Depending on the month the following differences in diversity and abundances were found for some of the analyzed groups: *Anchomenus dorsalis* was absent in March while it constituted the 17% of the total carabid captures in April and May. *Brachinus sclopeta* was absent in March and April while it was abundant in May representing 38% of the total captures of carabid beetles of the month. All individuals of the genus Harpalus were found in March and April (respectively 20% and 7% of all carabid beetles) but any *Harpalus* sp. was found in May. *Nebria salina* was present in the three periods but its proportion in March (20%) was higher than in the two following months (8% and 11%). *Poecilus cupreus* was

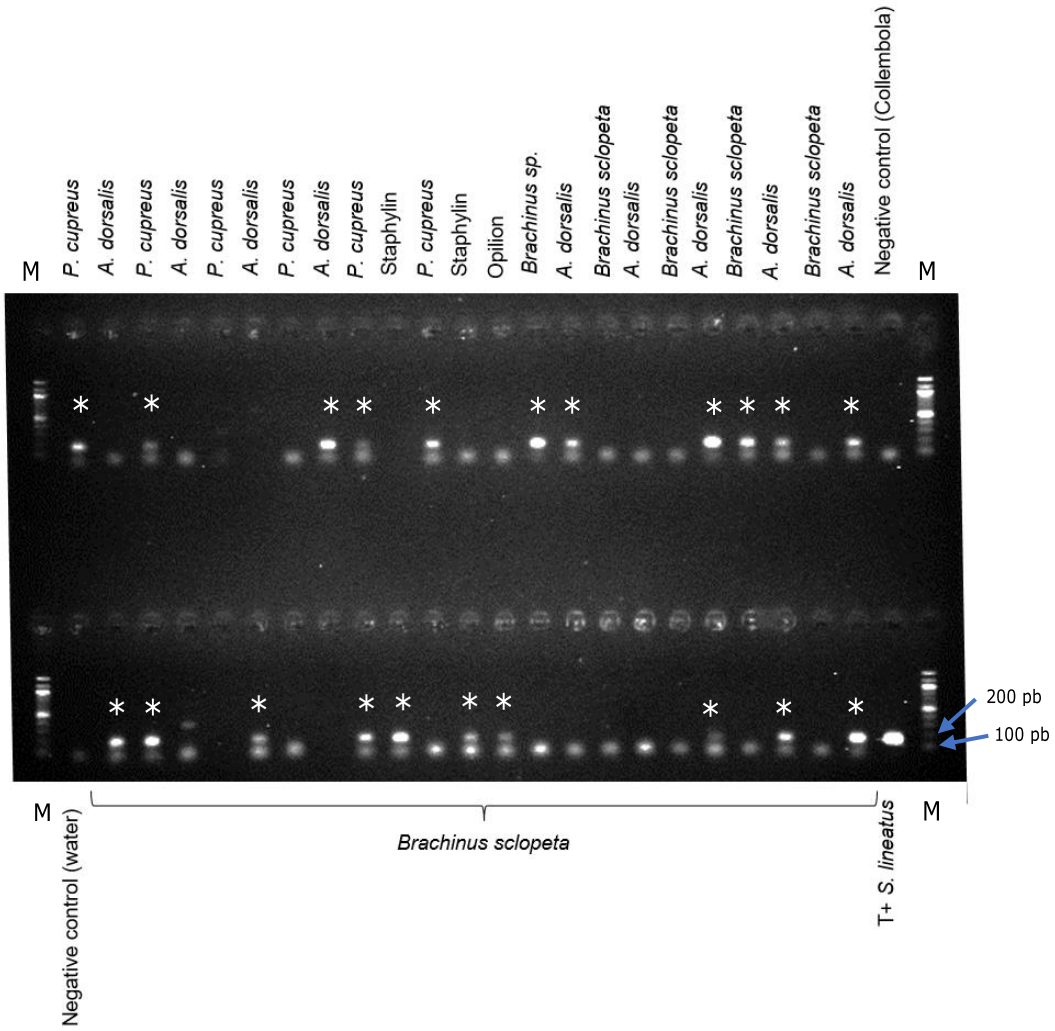

Figure 13: Agarose gel electrophoresis of PCR products when targeting *Sitona lineatus* DNA within predators' gut content. *=samples considered positive; lane M=DNA ladder.

Table 2: Groups of predator arthropods tested positive for *Sitona lineatus* DNA

Carabid beetles	*Amara* sp.	1	1
	Anchomenus dorsalis	16	58
	Brachinus sclopeta	11	25
	Brachinus sp. (other than sclopeta)	2	6
	Carabus nemoralis	1	3
	Nebria salina	6	23
	Nothiophilus sp.	5	5
	Poecilus cupreus	60	153
	Trechus sp.	6	7
Spiders	Family Gnaphosidae	4	5
	Family Linyphiidae	5	16
	Family Lycosidae	21	35
Rove beetles		10	25
Harvestmen		3	4
Centipedes		1	3
Earwigs	*Forficula auricularia*	1	5
Ladybugs	Family Coccinellidae	1	1

present and abundant in all capture periods showing a peak of abundance in April, with a 45% of all the carabid collected.

3.3. Screening of field-collected predators by molecular analysis

Out of the 558 samples screened in total, 142 were discarded for the analysis due to the two following problems: First, in one of the 96-well plates one of the negative control (water) was contaminated. Also, before extraction, when we submerged the tubes containing the samples in liquid nitrogen, some of them were damaged and they broke during the homogenization. As a result, we decided to eliminate of our analysis all suspicious sample in order to avoid false positives. Samples were considered positive when a band corresponding to the expected DNA size (151 pb) was present (fig. 13).

From the 416 arthropod predators successfully analyzed, 17 different taxonomic groups tested positive for the pea leaf weevil DNA (Table 2). In view of the preliminary nature of the results, rates of positive samples were only calculated for taxonomic groups comprising at least 20 individuals. Six taxonomic groups fall into this category: 4 species of carabid ground beetles, *Anchomenus dorsalis* with 27.6% of positive samples, *Brachinus sclopeta* with 44%, *Nebria salina* with 26,1% and *Poecilus cupreus* with 39.2%) as well as the rove beetle species (Fam. Staphylinidae) and the wolf spiders of the family Lycosidae, with positive rates of 40% and 60%, respectively (fig. 14). Nine other taxonomic groups showed positives but with limited sample size (Table 2). Concerning the 9 samples analyzed for the carabid ground beetles of the genus Harpalus (including 1 *Harpalus affinis* and 8 *Harpalus* sp., they all tested negative for *S. lineatus* DNA.

Summarizing, from the 416 predators tested, we found *Sitona lineatus* DNA in 154 samples, that being an overall positive rate of 37%.

3.3.a. Effect of intercropping on the rate of positives for *S. lineatus* DNA

When comparing the two types of studied plots, depending on the agronomical practice in which they were conducted (faba bean pure stand or intercrop with cereal), no significant difference could be found between the total rate of positives obtained in intercrop plots (103 positives out of 266 tested predators, that being a 38.72%) and from pure faba bean plots (51 positives out of 150 tested predators, or a 34%) (χ^2 p=0.3382).

At the taxonomic group level, the results were fairly stable with similar rates of positives from the intercrop catches and from the pure stands. The maximum rate was obtained with the family Lycosidae: 11 spiders (n=16) and 10 (n=19) were positives for pea leaf bettle DNA in intercrops and pure stands,

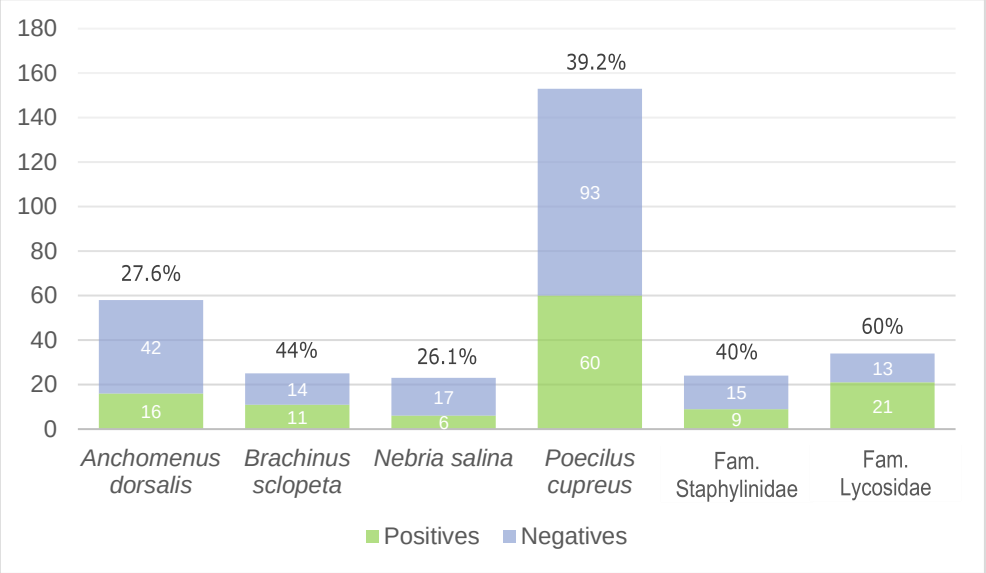

Figure 14: Number and percentage of individuals tested positive while targeting *Sitona lineatus* DNA in molecular gut-content analysis. Predators captured in different dates from March to May 2018.

respectively. *Poecilus cupreus*, the most abundant predator observed in our study, showed 48 (n=120) positives in intercrop and 12 (n=33) in pure stand.

3.3.b. Effect of plot area (edge or main crop) on the rate of positives for *S. lineatus* DNA

We also compared the number of predators tested positive for each of the two spatial zones studied in each plot (edge or main crop), finding that there was no significant difference between the total rate of positives corresponding to individuals captured within traps placed along the margin of the plots (96 positives out of 237 tested predators, that being a 40.5%) than for predators caught in the center of the plots (58 positives out of 179 tested predators, or a 32.4%) 5 (χ^2 p=0.0901).

Again, these results held true at the taxonomic group level. For example, 26 (n=64, 40.6%) and 34 (n=89, 38.2%) of the trapped *Poecilus cupreus* were positive for the pea leaf beetle DNA, respectively.

4. DISCUSSION

Our results indicate that *Sitona lineatus* individuals are actually being predated in faba bean fields of Western France. 37% of individuals belonging to several different groups of predator arthropods have tested positive for *S. lineatus* DNA detection within their gut-contents. This suggests a high level of pest predation to occur in faba bean fields. Our estimate might have been overestimated because a quarter of samples were discarded from PCR results in order to avoid false positives. However, when speculating that all these samples were negatives, the positive rate is corrected to 27.6%, which remains a high value. Conversely, our study focused on predators collected from the ground while many spiders were observed in the crop foliage where they could contribute significantly to pest adult predation. Taking these foliar spiders into account might have corrected upward the rate of positives.

Firlej *et al*. (2013) found that the most common carabid beetle in Québec soybeans, *P. melanarius*, tested positive for aphid DNA at the maximum rate of 33.7%. In Australian Brassica crops, Furlong *et al*. (2014) showed that spiders are the main predators of Lepidopteran pests with up to 23% and 38% containing prey DNA among Lycosidae and foliar-dweeling spiders, respectively. A similar study (Wolf *et al*., 2018) recently estimated that up to 40% of field-collected earwigs, spiders and bugs had consumed *Drosophila suzukii* in German fruit cultures. Hence, our estimate of the positive rate for pest DNA among arthropod predators compare well with previous studies in other agricultural systems. All these studies have in common that the pest was abundant throughout the cropping season. This held true for the pea leaf weevil in faba bean crops, as confirmed by our measurements of crop damages. We observed similar rates of positive samples among different taxonomic groups of carabids, spiders and rove beetles. We

also found, occasionally, positives among centipedes, harvestmen, earwigs and even a ladybug. All this makes *S. lineatus*, a very important food resource for generalist arthropod predators in faba bean fields.

Our observations of crop damages confirmed that the pea leaf weevil colonizes fields early in the season (Schotzko and Okeeffe, 1986). Hence, by providing food in early spring, this pest could increase the diversity and abundance of generalist predators in the crop. This could, in turn, favor the biological control of other insect pests found later in the season, such as the black bean aphid *Aphis fabae* which forms dense colonies affecting plant growth and the seminivorous bruchid species *Bruchus rufimanus*. It could be worth testing this hypothesis of an indirect positive effect of pea leaf weevil predation on the ecosystem service of natural pest control in faba bean fields.

Concerning intercropping, our findings confirm the hypothesis that this cultural practice has a positive effect on predator abundances, as we found a greater number of natural enemies in intercropped faba bean plots than in monocropping fields. Intercropping promotes plant diversity and niche theory implies that more heterogeneous habitats may support greater biodiversity through niche expansion (Weisberg *et al*., 2014). This explains why plant diversification is considered as a management strategy to promote the service of natural pest control (Symstad *et al*., 2000; Phillips and Gardiner, 2016). In our results, the effect of intercropping was especially noticeable for the four dominant species of carabid beetles: *Anchomenus dorsalis*, *Brachinus sclopeta*, *Nebria salina* and *Poecilus cupreus*. No conclusion can be drawn from the few preliminary data collected on the other carabid species. Interestingly, intercropping positive effect vanished for spider families, found in comparable abundances in the 2 cropping systems. This suggests that ground spiders are less restrictive for habitat quality than carabid beetles. However, what makes intercropped faba bean a more suitable habitat than pure stands for carabid beetles remains unknown. Our field network consisted in a majority of organic crops (12 fields out of 14) with a dense vegetation cover consisting of both cropped species (faba bean and cereal) and weeds. The only visible difference was the presence or absence of cereals which can hardly be connected to carabid abundance.

Intercropping effect on carabid community was restricted to predator abundance. Predator biodiversity measurements as inferred by the assemblage of taxonomic groups showed that the same communities were found in the two cropping systems. However, in our preliminary approach, we focused on the dominant species groups and we did not push specimen identification too far. More work is needed for describing the whole communities with less taxonomic redundancy. Last year, the implementation of a comparable protocol on the same study zone, revealed that the carabid diversity

(Shannon index) was higher in intercrops than in monocultures, with 37.5% and 10.6% of small carabid beetles (mean size inferior to 15mm), respectively (Albert *et al*., unpublished).

The spatial area within field showed a significant impact on spider and carabid beetles abundances as well. More predators were collected from the field edge than from the main crop. But the picture was really species-dependent, with specialists of the field edge (e.g. *Brachinus sclopeta, Lycosidae* spiders) or of the main crop (e.g. *Poecilus cupreus*) and generalist species (e.g. *Trechus*.sp). These results confirm the well-known foraging strategies of carabid beetles and spiders. Some species show marked preference for interface habitats, foraging the first meters inside the crop and the vegetation border during the same day. For many carabid beetle species (e.g. *Carabus nemoralis*), field borders constitute a network of corridors where they prospect, reproduce and disperse. They rarely leave their habitats to forage inside the crop. However, in intercropped fields with a dense vegetation cover, the transition between cropped and uncropped habitats may be less marked, enabling specialists of edge habitats to colonize the crop. This probably explains why, in our study, the 2 spatial areas shared almost all the taxonomic groups recorded and identified. Other studies (Pollier *et al*., 2018) showed that natural pest control varies with the distance to the field border, with generally less predators and predation toward the field center.

Interestingly, neither intercropping nor spatial area within field converted to any significant difference in the proportion of predators testing positive for pea leaf weevil's DNA. The effect of the spatial area in the field was only marginally significant. However, we cannot hypothesize that the level of pest biological control was independent of these 2 factors because we did not estimate the size of the pest population. For example, a similar rate of positives may result from a higher predation rate on a more abundant population. Modelling the relationships between crop damages and population size would help in understanding our results. Indeed, there is no clear correlation between the scale of leaves damages caused by adults and their population size (Cantot, 1989). Moreover, pest predation might be affected by predator interference when predators' abundance and diversity increase locally. Intraguild predation between natural enemies of the pea leaf weevil was observed in an experimental arena by Vankosky *et al*. (2011).

The predation of pea leaf weevils in our fields of study has been demonstrated, but the question of whether population dynamics are truly impacted remains difficult to answer. This is the same for the black box of the root damages. Females of the pea leaf weevil have a very high fecundity (Schotzko, D.J. and Okeeffe, 1986) which might compensate for significant levels of predation. Moreover, we still ignore what stages are consumed by arthropod predators. Vankosky *et al*. (2011) showed that some carabid species eat eggs of *S. lineatus*. But the exact diet of predators in field conditions and, more

specifically, the predation rate on eggs and adults remain unknown. Scavenging on dead adults by generalist predators may also occur and radically change the picture… Nonetheless, relating the highest rates of positives in PCR with the most abundant groups of predators in the fields we found that, *Anchomenus dorsalis*, *Brachinus sclopeta*, *Nebria salina* and *Poecilus cupreus* run into both categories at the same time. That clearly suggests that these species need to be seriously considered as important natural enemies of the pea leaf weevil and so, they deserve to be included in further studies about conservation biological control strategies.

PCR-based analysis can provide reliable accurate information of species identification and trophic links, but so far these techniques remain only qualitative and unable, on their own, to provide information for the understanding of the ecological significance of these relationships. Analysis giving a binary data set of results (predator testing positive or negative for prey detection), cannot provide quantitative information, but they can serve to produce semi quantitative data, like we did calculating the proportion of predators tested positive for *S. lineatus* DNA. Qualitative data are unable to provide information about how much amount of prey insect was consumed, or about the origin and nature of this detected genetic material (which was the ingested stage? egg, larva, nymph, adult?). Even when carrying out quantitative PCR analysis, from which we can be able to estimate the starting amount of DNA present in the sample, we can difficulty know with precision what happened between the predation event and the collection of the predator, because of DNA degradation processes. The proportion of prey remains detectable within predators, or rate of decay in detectability (Greenstone and Hunt, 1993), declines exponentially with time and is dependent on various factors, like the feeding mode and digestive physiology of the predator, the time from ingestion, the size of the target DNA fragment, or factors affecting the digestion process as temperature or the amount of non-target prey consumed by the same predator (Greenstone *et al*. 2007; Birkhofer *et al*., 2017). All these facts outline the importance of combining qualitative molecular gut-content analysis with quantitative ecological methods in order to assess the impacts of pests and natural enemy species in agro-ecosystems and identify which among them are actually key predators (Furlong, 2015).

Another important issue when performing PCR-based methods for gut-content analysis is the risk of obtaining false positives. This potential bias in the results could come from three main sources: first, a sample contamination; second, scavenging events; and third, the existence of intraguild predation. The risk of contamination between samples in this kind of studies, mainly coming from mass-collecting methods used for predators' catches, should not be disregarded (Greenstone *et al*., 2012). When collected, and inside traps, captured individuals could be externally contaminated through one (or more) of the following routes: direct prey released material, via predator regurgitates and via insect faeces (King *et al*., 2008; Greenstone *et al*., 2010). Using the protocol of sample preparation developed by

Wallinger *et al*. (2012) we made sure that no possible contamination would produce false positives in our results. Regarding scavenging events, it is known that carabid beetles and spiders both can have a scavenging behavior when food is limited. Even if in the fields of our study this didn't seemed to be the case (according to the assessment of the leaves damages, *S. lineatus* was always present and abundant in the crops) we must have this possibility into consideration. Testing scavenging in lab conditions might provide useful information for interpreting PCR results. Finally, concerning intraguild predation (IGP[5]), its prevalence among predatory arthropods is commonly accepted but its importance in our field conditions remains unknown. Small carabids are less concerned by IGP as they don't eat bigger predators. Hence, the rate of positives among these species may provide a better estimate of "real" pest predation in the fields. In our study, this rate was apparently independent of body size, suggesting that IGP did not add much to our global estimate of 37% positives.

5. CONCLUSIONS

We proved that different generalist predators, from seventeen taxonomic groups, are actually predating *Sitona lineatus* in faba fields of Western France. Combining PCR results with the assessment of species abundances, we showed that some of the most abundant species are also important predators of the pea leaf weevil, that leading us to the conclusion that this groups of predators should be taken into consideration for the development of future pest control programs.

We have also showed that our three factors of study, these being the type of cultural practice (intercropping or monocropping), the area of the plot were predators were collected (edge or main crop), and seasonality (captures in March, April or May) can have an influence on predator abundances. Concerning intercropping, the fact that this practice benefits natural enemies' abundances could also be taken into consideration when managing different type of pests.

Our results represent an outstanding first step towards the disentangling of trophic links between the pea leaf weevil and its natural enemies for the future design of effective appropriate management strategies in conservation biological control.

6. PERSPECTIVES

Concerning molecular analysis searching for *Sitona lineatus* DNA within predators, we have tested 416 samples for now. We have already accomplished significant findings, but the study is still in process, and we expect to analyze other 1400-1500 samples within the next two months. Once finished, the study will provide much more information for a more significant global interpretation of the results. The screening of a much greater number of samples would complete the present results and contribute to a better understanding of the trophic links found so far, and maybe also of new ones.

Furthermore, once all samples screened, we will send the DNA extracts to a different (and independent) laboratory for PCR confirmation (blind tests). Testing again the samples and comparing their findings with ours, we expect to run into the same results, in order to make sure that any handling error caused a bias in the results.

As well as for PCR analysis, the study to assess predator communities' biodiversity and abundances will also underway for the next two months. Thus, we will be able to classify all the specimens that wait for identification, after which we will finish some of the analyses that remain incomplete for now because of the lack of data.

Concerning the possibility of intraguild predation events (mentioned in the discussion) between the predators found positive for *S. lineatus* DNA, we could test that hypothesis using the same DNA extracts (those who tested positive for *S. lineatus*) and going one step forward in the PCR analysis. We could design a multiplex PCR approach, this time simultaneously targeting the DNA of those several groups of predators suspected to have participated in potential intraguild events. Then, we would analyze whether we detect or not the presence of some other species DNA in each of the samples. The same idea could be applied for searching, into those extracts, DNA remains of different preys (maybe some other pest) in order to know if they are also part of their diet.

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Sitography

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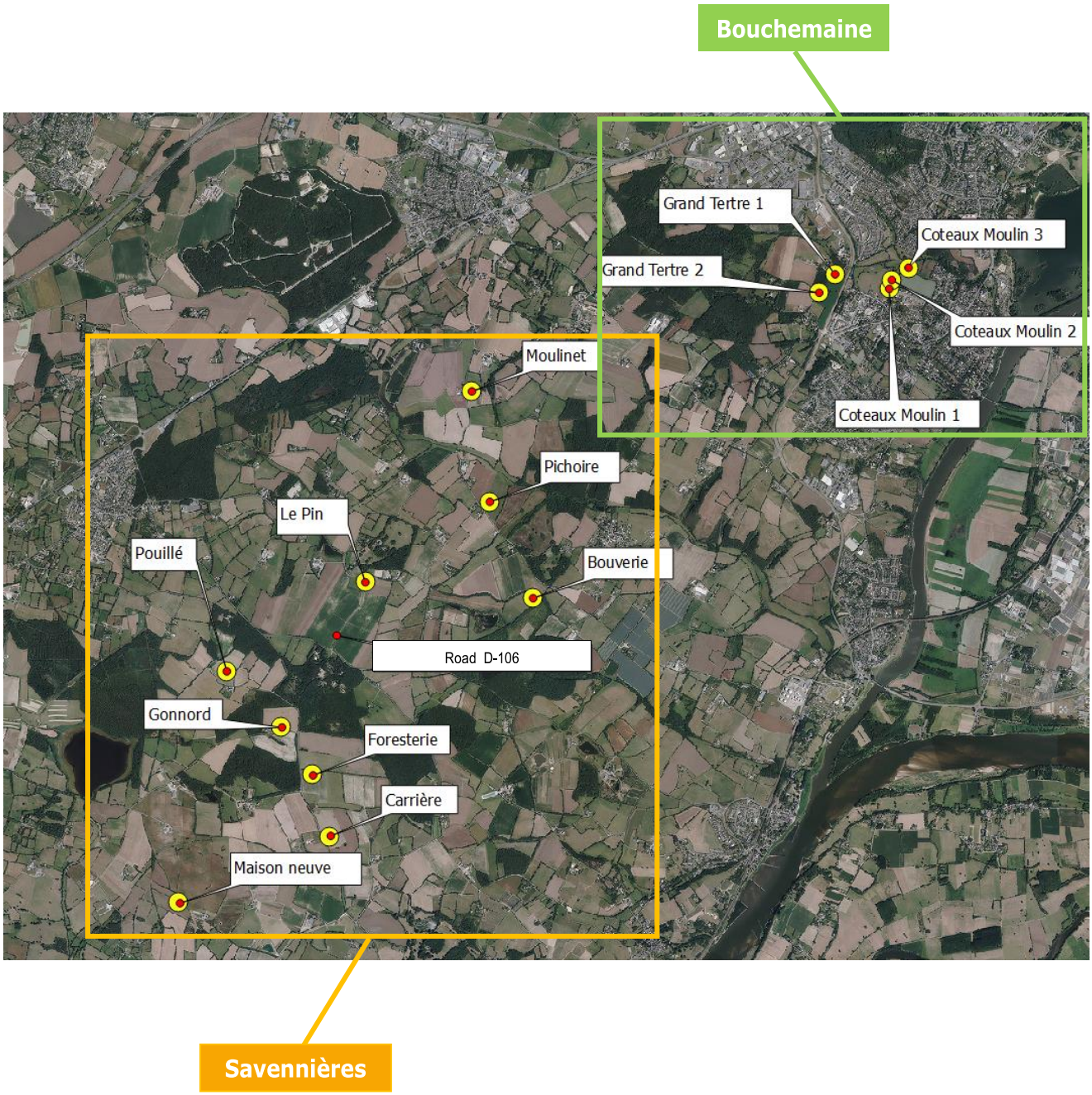
APPENDICES

Appendix I: Plot network map. Fourteen commercial fields in the municipalities of Savennières and Bouchemaine (Pays de la Loire; France).



María ROMA-MATEO, 2018. Biological control of the pea leaf weevil, *Sitona lineatus* (L.), in leguminous crops
Team IGEPP, UMR INRA 1349, Agrocampus Ouest / Site d'Angers, 2 rue André Le Nôtre F-49045, Angers, France.

ABSTRACT

In order to reach protein self-sufficiency, French livestock farming must develop sustainable soy cake alternatives such as local protein crops based on different leguminous like lupine (*Lupinus albus* L.), pea (*Pisum sativum* L.) or faba bean (*Vicia faba* L.). Among possible factors that put yields at risk, several insect pests (aphids, weevils and bruchids) may cause significant damages to the crops and the pea leaf weevil, *Sitona lineatus* L., is one of them. Feeding from root nodules, larvae can cause important crop yield reductions (Landon, 1995). From an agroecological approach, our study aims to contribute to the understanding of this pest dynamics and its interactions with its natural enemies in faba bean fields of Western France. First, we identified indigenous predators of the pea leaf weevil. Second, we evaluated the effect of intercropping on the natural predators' communities, from the hypotheses that mixed crops may improve the ecosystem service of biological control compared with pure stands. Pitfall traps were installed in 14 commercial fields (7 faba bean monocrops and 7 intercrop cereal/bean) for a mass-collection of arthropods, from which 1018 individuals served for the assessment of biodiversity, and 416 were tested by PCR amplification of *S. lineatus* DNA. Our results show a rate of 37% of the analyzed predators testing positive for *S. lineatus* DNA, from which 6 main groups presented significant rates of positives (between 26% and 60%): 4 carabid beetle species, rove beetles, and spiders of the Lycosidae family. Intercropping showed no significant difference in predator biodiversity, but a significant effect on arthropods abundances. Our findings provide useful information to the knowledge of the trophic relationships between *S. lineatus* and its natural enemies, which can be used to the development of future efficient conservation biological control strategies.

Key words : Agroecology, Conservation biological control, Predation, PCR, pea leaf weevil

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María ROMA-MATEO, 2018. Contrôle biologique du sitone du pois, *Sitona lineatus* (L.), en cultures de protéagineux
Team IGEPP, UMR INRA 1349, Agrocampus Ouest / Site d'Angers, 2 rue André Le Nôtre F-49045, Angers, France.

RESUME

Dans le but d'atteindre l'autonomie protéique, l'élevage français doit développer des alternatives durables au tourteau de soja, tels que des protéagineux locaux comme le lupin (*Lupinus albus* L.), le pois (*Pisum sativum* L.) ou la féverole (*Vicia faba* L.). Parmi les facteurs qui peuvent impacter négativement le rendement de ces cultures, plusieurs insectes ravageurs (pucerons, sitones et bruches) peuvent provoquer des dégâts significatifs et le sitone du pois, *Sitona lineatus* L., est un d'entre eux. En se nourrissant des nodosités racinaires, les larves de *S. lineatus* sont capables d'engendrer des pertes de rendement importantes (Landon, 1995). Dans le cadre d'une démarche agroécologique, notre étude vise à contribuer à la compréhension de la dynamique ce ravageur et à ses interactions avec ses ennemis naturels dans les champs du Nord-Ouest de la France. Pour ce faire, nous avons cherché à identifier les prédateurs indigènes des sitones. Nous avons également testé l'effet des associations culturales sur les communautés naturelles de prédateurs, en formulant l'hypothèse que cette pratique bénéficie au service de contrôle biologique, comparativement à des parcelles en monoculture. Des pièges Barber ont été installés dans 14 parcelles (7 en monoculture et 7 en association céréale/féverole) pour capturer des arthropodes, à partir desquels 1018 individus ont servi pour l'étude de biodiversité et 416 ont été analysés par PCR en ciblant l'ADN de *S. lineatus*. Nos résultats montrent une proportion de 37% de prédateurs positifs à l'ADN de *S. lineatus*. Parmi eux, 6 groupes principaux ont montré des proportions de positifs importantes (entre 26% et 60%) : 4 espèces de carabe, les staphylins et les araignés de la famille Lycosidae. L'effet des associations culturales a été constaté sur les abondances de prédateurs mais pas sur leur diversité. Nos résultats fournissent des informations utiles pour la compréhension des liens trophiques entre *S. lineatus* et ses ennemis naturels qui peuvent être utilisées pour le développement de futures stratégies de lutte biologique par conservation.

Mots clés : Agroécologie, Lutte Biologique par Conservation, Prédation, PCR, sitone du pois