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AGROECOLOGICAL MANAGEMENT OF INSECT PESTS ON SUGARBEETS AND LETTUCE CULTIVATED IN ORGANIC FARMING

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Abstract

Two-year field trials were conducted in northern Italy with the aim of developing a trapcrop-based agroecological approach for the control of flea beetles (*Chaetocnema tibialis* (Illiger), *Phyllotreta* spp. (Chevrolat) (Coleoptera: Chrysomelidae)) and *Lygus rugulipennis* Poppius (Hemiptera: Miridae), key pests of sugar beet and lettuce, respectively.

The flea beetle damage trials compared a trap cropping treatment, i.e., a sugar beet plot with a border of *Sinapis alba* (L.) and *Brassica juncea* (L.) with a control treatment, i.e., a sugar beet plot with bare soil as field border. Sugar beets grown near trap crops showed a significant decrease (≈40%) in flea beetle damage compared to control. Moreover, flea beetle damage varied with distance from the edge of the trap plants, being highest at 2 m from the edge, then decreasing at higher distances.

Regarding *L. rugulipennis* on lettuce two experiments were conducted. A semiochemical-assisted trap cropping trial was supported by another test evaluating the efficacy of pheromones and trap placement. In this trial, it was found that pheromone baited traps caught significantly more specimens of *L. rugulipennis* than unbaited traps. It was also found that traps placed at ground level produced larger catches than traps placed at the height of 70 cm. In the semiochemical-assisted trap cropping experiment, a treatment where lettuce was grown next to two Alfa-Alfa borders containing pheromone baited traps was compared with a control treatment, where lettuce was grown near bare soil. This experiment showed that the above-mentioned strategy managed to reduce *L. rugulipennis* damage to lettuce by ≈30%.

From these studies, it appears that trap crop-based strategy, applied alone or with baited traps, made it possible to reduce crop damage to economically acceptable levels and to minimize the need for specific insecticide treatments, showing that those strategy could be implemented in organic farming as a means of controlling insect pests.

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1.1. Economic, Descriptive and Market Data on The Production of Sugar Beet

Sugar beet (*Beta vulgaris* L.), together with the sugar cane (*Saccharum officinarum* L.), are the two main crops, used for the industrial production of sucrose (Erdal *et al*., 2007; Zimdahl R.L., 2004). Sugar beet is grown in 53 countries and provides about 12% of the total world sugar production. It is also important as a source for bioethanol and animal feed. (FAO, 2022).

In the year 2020, sugar beet was cultivated on 4,439,073 hectares for a total production of 252,968,843 tonnes (FAO, 2022). Of the global sugar beet production, Europe contributes for a 62.1 %, with 157,098,827 tonnes produced, followed by Asia (18.3 %, 46,219,276 tonnes), Americas (13 %, 32,881,251 tonnes) and Africa (6.6 %, 16,769,489 tonnes) (FAO, 2022).

Nowadays, Russian Federation (RF) is the biggest producer of sugar beet, with a harvested area of 916,647 hectares, which is nearly 20% of the global hectares sown with the crop. RF production, indeed, reaches the 13.41 % of the global sugar beet production, with 33,915,086 tonnes. The other top sugar beet producers are United States of America (with 30,497,740 tonnes), Germany (with 28,618,100 tonnes), France (26,195,460 tonnes), Turkey (with 23,025,738 tonnes), Poland (with 14,171,540 tonnes), Egypt (with 13,043,612 tonnes) and China (with 11,597,764 tonnes) (Fig.1).

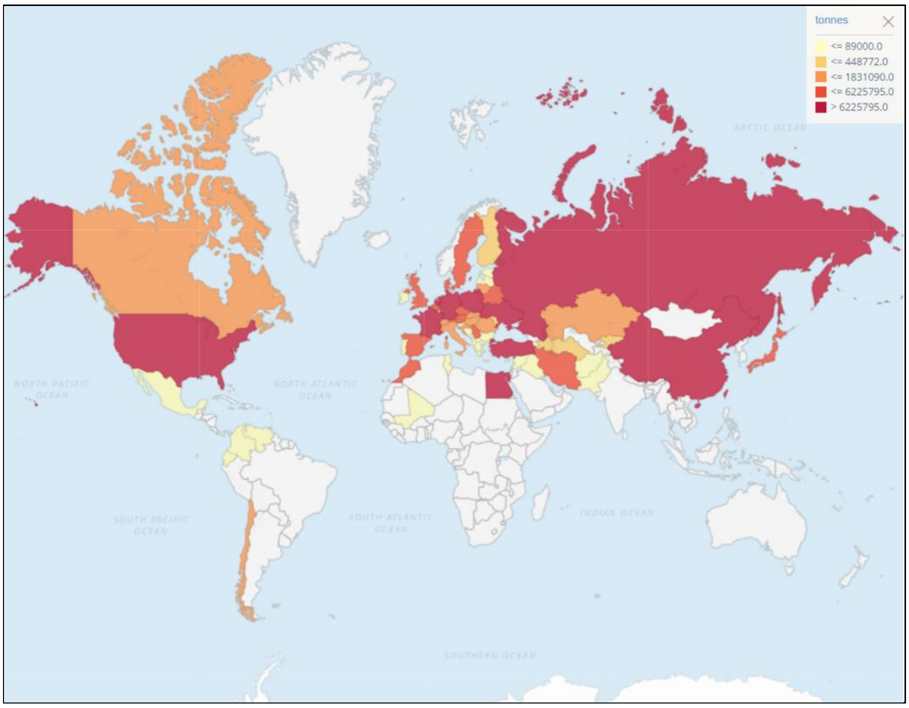

Figure 1 - Global sugar beet production by country (Faostat, 2022).

For years, among European countries, Italy has been one of the top 10 sugar beet producers but suffered from a severe blow from the European Union Community reform of the sugar sector which began in 2006, that led to a decrease of approximately 60 % of sugar beet production in 14 years (FAO, 2022). This reform placed a maximum production quota intended for consumption to each European country: the part of production exceeding this quota had to be exported, used as biofuel or for other non-food industrial purposes, or subtracted from the following year's quota. The main goals of the reform were to prevent price of European sugar from dropping too much, to increase sugar sector concentration (Aragrande *et al*, 2017), and to create few large producers able to compete without subsidies on world markets; in Europe, indeed, before the reform, sugar production was spread in 23 Member States, while, after the reform, production was concentrated in the 18 Member States that presented the most favourable agronomic conditions. Subsequently, in 2017, the end of the quota system, which managed sugar in the EU, led to a liberalization of sugar production, and consequently also of sugar beet production, which caused sugar beet prices to collapse: from 600 to 370 €/ton.

In Italy, this has led, over the last decade, to a drastic decline of the areas destined for sugar beet cultivation, and consequently also of its productions (Fig. 2), and also to a decrease in the number of active sugar refineries from 19 to just 2.

In Italy, sugar beet is nowadays harvested on 27,270 hectares, for a total production of 1,831,090 tonnes (year 2020) (FAO, 2022).

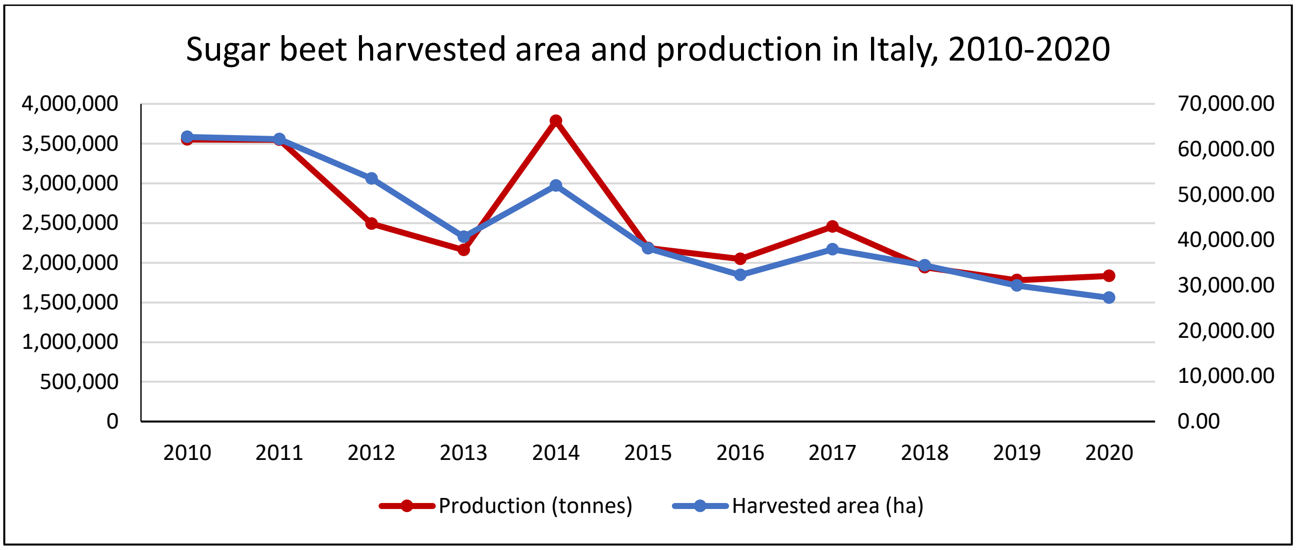

Figure 2 - Italian sugar beet harvested area and production (Faostat, 2022).

1.1.1. Insect pests and diseases

Sugar beet is a crop that has a long growing season, up to 200 days, during which it may be exposed to several diseases, including insect pests, nematodes, fungal diseases, and viruses (Yamane, 2016).

Sugar beet is attacked by many arthropod pests, which may cause direct injury or may introduce virus diseases to the plant, causing severe economic loss (Baker, 1975). These harmful insect pests, on sugar beets, are generally framed according to the growth phase in which the plant is attacked. The most critical period for arthropod pest damage is early in the season when the plants are at the seedling stage (sugar beet are considered to be "seedling stage" up to about four rough-leaf stages) (Meier, 2018). As seedlings, sugar beet can be attacked by various phytophages, some parasites of the underground parts, other parasites of epigean parts.

Among the first, wireworms, larvae belonging to the Elateridae family, can certainly be considered among the most dangerous soil pests of a wide range of arable crops in Europe, including sugar beets (Veres *et al*., 2020; Jansson and Seal, 1994; Parker and Howard, 2001; Vernon *et al*., 2005). The most harmful species in Europe belong to the genus Agriotes Eschscholtz. In Northern Italy, *Agriotes brevis* Candèze, *A. litigiosus* Rossi, and *A. sordidus* Illiger (Elateridae: Elaterinae: Agriotini Champion, 1894) represent the most widespread species (Furlan and Tóth, 2007; Furlan *et al*., 2001; Furlan *et al*., 2017).
Their life cycles last 1-5 years (Furlan, 2004), where the larval stage (the so-called wireworms) represents the main overwintering stage, if not the only one. These *Agriotes* species, indeed, fall into two main groups: species that are able to overwinter also as adults (like *A. sordidus* and *A. brevis*), and species that cannot overwinter as adults (like *A. litigiosus*) (Furlan, 2005).
The only stage dwelling outside the soil is represented by the adult stage, which does not damage to crops, while the larval stage, which is the only harmful stage, lives underground.
Wireworms are very polyphagous pests that feed on seeds, roots, stems, tubers, and belowground plant parts (Keiser *et al*., 2012; Traugott *et al*., 2015). On sugar beet, their attacks are very dangerous especially on the first development stages of seedlings, as they feed on the first roots, near the collar, thus inhibiting plant growth, causing plant wilting and death, subsequently reducing crop yield. Wireworms are very difficult to control since it is impossible to know exactly their position and the population present in the field without carrying out specific excavations or monitoring with food traps. Anyway, strategies aiming at reducing wireworm densities below the

economic threshold (available on maize but not yet on sugar beet) should integrate more than one practice with a partial impact. Among preventive practices, crop rotations unfavourable to wireworm survival and oviposition, frequent tillage (wireworms are extremely sensitive to drought and high temperatures so tillage would expose them to more unfavourable conditions), the incorporation of plants with biofumigant properties into soil, and the use of natural enemies for pest control can be listed (Poggi *et al*., 2021).

Among early sugar beet stages pest of epigean parts, flea beetles (which will be deepened in Chapter 1.2) and Atomaria can be counted.

The pygmy mangold beetle, *Atomaria linearis* Stephens, is a small dark-brown beetle, 1 mm long, native to Europe (Johnson, 1992). It is widespread throughout all European countries, but it is a serious pest especially in central-eastern regions (Muška, 2007), where it finds its best habitat conditions in clayey and humid soils and temperatures between 10 and 20 °C (Cochrane and Thornhill, 1987).

Given its optimum climatic conditions, this pest attacks sugar beet seedlings in the early spring, when they are very susceptible. *A. linearis* damages sugar beet by making circular erosions at collar level of the seedlings, about 1 mm below ground level, causing them to collapse and to failure in the fields. More rarely it erodes leaves and the hypocotyl with a diameter of 0.5-2 mm, causing the tissues to necrotize with the consequent appearance of bottlenecks that make plants wither and die. The containment of the infestations is mainly based on good agronomic and agroeological techniques, most important of which is the crop rotation and the avoidance of crop re-growth or too short rotations.

In the following vegetative phases, already developed sugar beets can be damaged both in the taproot and in the foliar system. Taproot attacks are normally caused by Curculionid beetles, while leaves are mainly affected by aphids and Lepidoptera Noctuids.

Among Curculionid beetles, the western sugar-beet weevil *Conorrhynchus* (*Cleonus*) *mendicus* Germar and *Lixus juncii* Boheman are the most harmful species that can be found damaging sugar beets in Italy.

Conorrhynchus mendicus is an important pest of sugar beet throughout the entire southwestern part of Europe (Hoffmann, 1966), where it can severely harm the plants in both adult and larval stages. Adults, light or dark grey and 11 to 17 mm long, after overwintering, start feeding on young sugar beet plants, reducing crop stand at high population levels.

Contrariwise, larval stages, which live underground, dig tunnels inside the sugar beet taproot mainly during late spring and summer, significantly reducing both the quantity (for direct damage) and the quality (settlement point for cryptogamic rottenness) of the yield (Campagna, 1999; 2001; 2002). Against *C. mendicus*, control measures consist mainly in preventive planning of sugar beet field location, since an inverse relationship between colonization rate of adults vs distance from overwintering sites and sugar beet fields has been demonstrated (Burgio *et al*., 2000) and trapping, which is generally carried out by using pitfall traps, made up of plastic buckets placed in the soil, into which crawling beetles fall and get caught. In the presence of heavy infestations, starting from more than 3 adults caught/trap/week, specific insecticide treatments are required, generally carried out with permitted pyrethroids.

Lixus juncii, also known as sugar beet weevil, is, into the genera Lixus, which includes up about to seventy species, the most common and harmful species found damaging sugar beet in the Mediterranean area and in Italy (Isart, 1966; Ocete *et al*., 1994). It is a highly polyphagous species, especially present where sugar beets, Chenopodiaceae (e.g., *Atriplex halimus*, *Atriplex hastatum*, *Spinacia oleracea*) and Cruciferae (*Brassica campestris* and *Brassica oleracea*) grown or are cultivated. The adult stage presents a variable length between 10-15 mm, with a dark grey or blackish colouration, with a white border under the elytra, a characteristic that distinguishes this species from other *Lixus* weevils.

Lixus juncii, after the overwinter (which is carried out into the ground at the adult stage), between April and May gradually migrate into new sugar beet fields in order to feed on the seedlings and to lay their eggs at the collar of the young plants or within the leaf peduncles. Unlike *C. mendicus*, *Lixus juncii* adults do not cause particular damage on the leaves of young seedlings, as they feed mainly on spontaneous species before arriving on sugar beets to mate. Each female lays an average of 40 to 50 eggs. After about ten days after oviposition, eggs hatch and new-born larvae immediately descend into the taproot by digging vertical tunnels that fill with their metabolic residues. The cycle is completed inside the taproot from early summer month until sugar beets harvest. *L. juncii* makes only one generation a year. Damage is determined mainly by direct larval feeding activity, which lowers root yield. In second place, galleries dug can become a settlement point for cryptogamic rottenness, lowering both yield and quality of taproots.

Lixus juncii control is very difficult, since once oviposition has taken place, there are currently no known insecticides capable of effectively killing larvae within petioles or taproot. The control is oriented, through visual monitoring of infestations in the field, towards choosing the best time to carry out specific insecticide treatments on adults in the mating phase. Adult and larvae parasitoids are also reported. They are represented mainly by the Diptera Tachinidae: *Rondania cucullata*

Robineau-Desvoidy (on the adults) and *Zeuxia cinerea* Meigen (on the larvae). Other parasites reported are the hymenoptera *Pimpla roborator*, *Microbracon intercessor, Eurytoma curculionum*.

Among pests of the leaf system on developed sugar beet plants, aphids certainly represent those that concern farmers more because of both damage they directly cause to plants, but more because of act as a vector for the virotic yellows. Black bean aphid (*Aphis fabae* Scopoli) and green peach aphid (*Myzus persicae* Sulzer) are the two main aphid species found damaging sugar beet (Dewar and Cooke, 2006; Dewar, 2007). The black bean aphid has dark body, around 1.5 mm long, while green peach aphid body is yellow-green and teardrop-shaped, around 2 mm long. Both aphids overwinter as eggs on their primary host, respectively some spontaneous species (e.g., *Viburnum spp*. and *Euonymus spp*.) for *A. fabae* and peach or other stone fruits for *M. persicae* (Ferrari *et al*., 2006). On these primary hosts they complete few spring generations, from March to May, before gradually moving on to the secondary guests, which include sugar beet. Damage on sugar beets is partially due to direct damage, caused by the pricking on the leaves and the injection of saliva, followed by the sucking of the vegetable juices. Leaves, if intensely affected, take on a rippled appearance with crumpled edges. Plants attacked by aphids are also threatened by the aphids abundant production of honeydew that smears the organs, causing partial asphyxiations as well as favouring the development of black, sooty mold to cover the leaves and reduce plant photosynthetic ability. As already mentioned, however, the greatest damage derives from the aphid's ability to transmit virosis (indirect damage). Both aphid species are capable of transmitting Beet mosaic and yellowing virus (BMV), even though black bean aphid is less dangerous as virus vector than green peach aphids.

In general, small aphids infestations are well controlled by the natural enemies of the aphid such as many Ladybirds, Hoverflies, Chrysopids and various Hymenoptera. However, if heavy infestations occur, specific aphidic treatments are required.

Finally, on developed sugar beet, several Lepidoptera, belonging to the family Noctuidae can be found damaging leaves. Among these, *Autographa gamma* L., *Mamestra brassicae* L., *Mamestra oleracea* L. and *Spodoptera exigua* Hübner are considered the most dangerous.

A. gamma is a very polyphagous pest; more than 200 host plant species are reported (Nash and Hill, 2003). It is widespread throughout Europe, Asia, and Northern Africa (CABI, 2007). One of the main host plants is represented by sugar beet, on which the larvae cause economic losses by feeding on leaves, and subsequently reducing yields.

M. brassicae and *M. oleracea* are known pest responsible for severe crop damage of a wide variety of plant species (Castellari, 1968). In particular, *M. brassicae*, whose common name is cabbage moth, feeds on many fruits, vegetables, and crops belonging to the genus *Brassica* (e.g., cabbage, broccoli, brussels sprouts) (Wu *et al*., 2015). *M. oleracea* also show preference for Brassicaceae crops, as well as Chenopodiaceae and Papilionaceae. Likewise *A. gamma*, *M. brassicae* and *M. oleracea* damage is delt by larvae. Indeed, once the eggs have hatched, new-born larvae live on the lower page of the leaves, at the expense of which they feed by gnawing the epidermis and the parenchyma, generally respecting veins, and petioles.

The beet armyworm, *S. exigua*, is a phytophagous pest that has a wide host range and feeds on more than 170 plant species, including sugar beet (Zhang *et al*., 2011; Goodarzi *et al*., 2015). Similarly to other Noctuidae, damage on sugar beet is caused by larval intense feeding on leaves, which can lead to significant yield loss. Moreover, *S. exigua* larvae feeding on the taproots near the soil opens the way for the entry of pathogens which cause heavy loss.

To control these pests there is a wide range of effective and specific insecticides. It is important to alternate them in order to limit the reported phenomenon of resistance (Moulton *et al*., 1999), and to correctly time treatments against the first generations, since each female will give rise to hundreds of individuals.

Among fungal diseases, Cercospora leaf spot and Sugar beet root rots are the most dangerous and can cause severe economic damage if not properly controlled. Indeed, Cercospora leaf spot (CLS), caused by the hemibiotrophic fungus *Cercospora beticola* Sacc., is the primary foliar disease of sugar beet worldwide and can cause yield losses around 20 % every year (Weiland and Koch, 2004; Jacobsen and Franc, 2009; Khan *et al*., 2009; Secor *et al*., 2010). CLS infection symptoms generally present as necrotic purple or red-brown round spot lesions of initially 0.2–0.5 cm in diameter across the surface of mature leaves, subsequently coalescing into leaf necrosis, which lowers the plant's photosynthetic ability and reduces sucrose production and root growth (Harveson, 2013; Duffus and Ruppel, 1993). The control of this disease in the field has historically relied on the use of copper. However, recently, following the limitations on the quantities of copper to be used in agriculture, more reliance is placed on interventions with synthetic chemical fungicides, mainly based on Tetraconazole, Prochloraz, Difenoconazole and Fenpropidin. Moreover, to reduce the use of these chemical products, and consequently their environmental impact, their application is nowadays guided by specific forecasting models. These models have been developed thanks to the understanding of the optimum environmental conditions for the beginning of *C. beticola* epidemics (elevated temperature, humidity, and leaf wetness), and are capable of predicting the onset of the disease in the field (Bleiholder and Weltzien, 1972; Pool and

McKay, 1916; Shane and Teng, 1983). As for an agronomic method to control the disease, there are some sugar beet varieties that are resistant or tolerant to *C. beticola*. These varieties, however, have historically shown a lower yield than traditional susceptible varieties but, nowadays, yield performance of recent varieties with resistance to *C. beticola* caught up with susceptible varieties due to breeding progress (Vogel *et al*., 2018).

Damping-off and root rots, mostly caused by soil-borne fungi and some bacteria, occur in almost all of the sugar beet production areas of the world. Among the root rot pathogens, *Aphanomyces cochlioides* Drechsler and *Rhizoctonia solani* Kühn represent the most important disease complex on sugar beets, but *Phoma betae* Björl, *Pythium spp*., and *Phytophthora drechsleri* Tucker have also been listed as potential agents of sugar beet damping- off and root rot in many sugar beet production areas (Jacobsen, 2006; Harveson *et al*., 2009).

Aphanomyces cochlioides, which is the causal agent of black root rot, is found in several sugar beet growing areas of USA, Canada, England, Europe, Chile, and Japan. For the infection and development of the fungus warm temperature (between 22-28°C) and wet soils are required. (Jacobsen, 2006). Depending on the environmental conditions and the quantity of soil infestation, economic losses can be up to 100% (Windels and Brantner, 2000). *A. cochlioides* is able to cause both a chronic seedling disease (known as black root) and a chronic root rot phase. Black root symptoms usually start with the appearance of greyish, water-soaked lesions on the stems near the soil level, turning darker over time and extending upward the stems, causing them to turn black. Root rot symptoms, instead, begin as yellow brownish, water-soaked lesions, which extend inside the root, becoming dark brown or even black as the disease progress. It usually occurs as tip rot but can occur anywhere on the root.

Rhizoctonia root and crown rot (RRCR), which is caused by the fungus *Rhizoctonia solani* Kühn AG 2-2, is a widespread disease wherever sugar beet is grown, and has spread over a large part of Europe and USA in the last decades (Büttner *et al*., 2004; Märländer *et al*., 2003; Liu *et al*., 2019). Indeed, it is present in more than 25% of the sugar beet production area of the USA and in 5-10% of Europe (Windels *et al*., 1997; Haverson, 2008).

Rhizoctonia solani is an endemic, soilborne pathogen that lives in soil independently of the host presence, competing with the microflora and depending on host plant and on environment only to propagate over space and time (Anees *et al*., 2010). RRCR is generally correlated with the development of the vegetation on the plant; as a result, it mainly occurs towards the end of the season and on older plants (Hillnhütter *et al*., 2011). On infected plants, starting from the petioles in contact with the ground, black lesions appear at the base; subsequently, rot spreads to crown and roots. As the disease progresses, the first symptoms appear also on the epigeal apparatus, which include severe wilting, collapse and yellowing of leaves. Root rot then develops, forming circular black lesions, which often clump together to cover large root surface areas (Herr, 1996; Windels and Nabben, 1989).

Severity of the disease has been positively correlated with favourable temperature conditions (optimum temperature for pathogen growth is 25-28°C) and with irrigation (higher soil moisture) (Baker and Martinson, 1970; Rush and Winter, 1990; Windels and Brantner, 2000).

Worldwide, significant economic losses are caused by RRCR, depending on the extent of the disease attack, and varying from field to field, reaching up to a 60% yield depletion (Bartholomäus *et al*., 2017; Allen *et al*., 1985; Buhre *et al*., 2009).

Rhizoctonia solani disease management on sugar beet includes fungicide application during seed treatment or at 6-8 leaf stage in some countries, and crop rotation but, since the pathogen is endemic in all sugar beet growing areas and is a soil inhabitant, these measures are of minor value in control (Whitney and Duffus, 1986). Resistant or tolerant cultivars to Rhizoctonia are present but are usually correlated with lower yield, from -10 to -15% compared with susceptible varieties (Panella and Ruppel, 1996).

Another important fungal disease that, if not properly treated, is able to cause yield losses, up to 30 % on sugar beet, is powdery mildew, caused by *Erysiphe betae* (Francis, 2002). Nowadays, powdery mildew can occur in almost all sugar beet growing areas, but it is better adapted to environmental conditions of semi-arid regions with warm, dry climates and large diurnal temperature fluctuations (Neher and Gallian, 2013). *E. betae* infections typically begin on older leaves, mainly on the junction between lamina and petiole. The first symptoms present as small, scattered, circular, white dust-like mycelium colonies that grow over both leaf surfaces. Under favourable environmental conditions the disease progresses, and the colonies coalesce infecting all the leaves, making the plant take on a dusty white appearance. Heavily infected tissues develop chlorosis and suffer early senescence. This disease is commonly controlled by using sulphur-based fungicides, the usage of which started in the 1970s against powdery mildew and has been increasing since then (Byford, 1996).

Among other significant sugar beet disease, Rhizomania plays a particularly important role. Rhizomania disease was discovered in Northern Italy in 1959 by Canova. He witnessed occurrences of poorly growing sugar beet crops, naming their condition “root madness” since the presence of an abnormal proliferation of dark necrotic roots (McGrann *et al*., 2009). The cause of the disease, however, remained uncertain until 20 years later, when, in Japan, a virus, named Beet necrotic yellow vein virus (BNYVV), was isolated from infected sugar beet plants (Tamada and Baba, 1973). BNYVV is transmitted by the widely spread soilborne protoctist *Polymyxa betae* Keskin (Fujisawa and Sugimoto, 1976). Nowadays Rhizomania is a serious threat throughout the major sugar beet growing regions of the world, causing severe yield losses in the absence of effective control measures (Tamada, 1999; Lennefors *et al*., 2005). The first symptoms of Rhizomania disease in sugar beet occur with light-green or yellow patches in the field, usually early in the growing season (Pavli *et al*., 2011). On roots, Rhizomania symptoms include characteristic proliferation of fibrous secondary and tertiary roots around the tap root, that eventually become necrotic and give the root a bearded appearance (Richard-Molard, 1985; Putz *et al*., 1990).

Sugar beets is also severely threatened by nematodes. In particularly, the beet cyst nematode (BCN), *Heterodera schachtii* Schmidt (Schmidt, 1871) causes major yield losses in sugar beet and other crops worldwide. It has been acknowledged as pathogen of plants in 1859 in Germany (Schacht, 1959), but it is now widespread in most of the beet-growing areas in the world, causing yield losses up to 60%. Even though its name, it has a very wide host range outside sugar beets; it is able, indeed, to infect more than 200 plants, mostly of which are plants of the families Amaranthaceae (many species of *Beta* and *Chenopodium*) and Brassicaceae (Franklin, 1972). *Heterodera schachtii* is a parasitic roundworm. Its life cycle begins with the hatching of the eggs inside the cyst, which is the body of a dead female. The new-born juveniles remain dormant inside that cyst until they come in contact with a root of a host plant which has grown near the cyst. Then, if the soil moisture is adequate, juveniles are stimulated by root exudates to emerge from the cyst. Subsequently, juveniles infect fibrous roots near the root tips, where they enter to develop into adults. Adults become sedentary and will undergo a series of multiple moults. After several moults, adults emerge from the root and enter the soil, where female lay their eggs. A female, generally, is able to lay up to 200 eggs, some of which are laid in the soil, but the majority of the eggs remain inside the body of the female. Once reached maturity, the female dies, and her body hardens and transforms into a brown-reddish cyst, completing the cycle. To complete the cycle, four to six weeks are required, depending on soil temperature.

Heterodera schachtii is able to parasitize sugar beet roots of different ages. The most dangerous damages are those to sugar beet seedling, that may even be killed, with the result of lower plant densities. If the infection occurs on a young plant, it may develop elongated petioles and remain stunted until harvest time. Infected plant, generally, present wilted leaves, especially during the hottest hours of the day or with low soil moisture. Leaves can also have pronounced yellowing, well visible in the field. Regarding underground system, infected plants present small taproots, which are severely branched with excess fibrous roots often referred to as "bearded" (Biancardi, 2010). These symptoms are generally less noticeable when older plants are infected. Damage to plants is greatest in a dry summer, when plants are stressed, following a wet spring, which is conducive to nematode infection.

To control sugar beet nematode, one of the most effective methods is the rotation with a non-host crop, which may include wheat, barley, maize, and alfalfa. Also, the early sowing, carried out when soil temperatures are relatively cool, may help in reducing damage, since plants can grow when the nematode is still underactive to better tolerate its attacks in an older age. For sure, the most effective and economic approach to control *H. schachtii* on sugar beets is growing tolerant or resistant cultivars. Many of those cultivars, when infected by juveniles of *H. schachtii*, which can establish a feeding site, its syncytia degenerate before nematode maturation, hence deterring lifecycle competition (Cai *et al.*, 1997, Yu and Steele, 1981).

1.2. Flea beetles (Chrysomelidae: Alticini)

Flea beetles are small, jumping beetles, which belong to the leaf beetle family (Chrysomelidae), to the subfamily Galerucinae and to the tribe Alticini Spinola, which counts more than 80 genera. Of these, species belonging to genus *Chaetocnema* Stephens, *Altica* Geoffroy, and *Phyllotreta* Chevrolat in Dejean are the ones most often found to cause damage to sugar beet. In particular, the main *Chaetocnema* species present in northern Italy environment is *Chaetocnema tibialis* Illiger (Biondi, 1990a; 1990b), while among genera *Altica* and *Phyllotreta* several species are greatly widespread in northern Italy.

1.2.1. Morphology

Chaetocnema tibialis adult has a body length of up to 2 mm and a width of around 1 mm. Body ratio are reported in literature (Konstantinov *et al.*, 2011). The ratio of elytra length at suture compared to maximum width is 2.56-2.82. The ratio of pronotum width at base compared to length at middle is 1.56-1.85. The ratio of elytra length at suture compared to length of pronotum at middle is 3.02-3.45. The ratio of both elytra's width at base compared to pronotum width at base is slightly over 1.1. The ratio of both elytra's maximum width compared to pronotum maximum width Is about 1.5. This pest's body colour is, therefore, mainly given by elytra's colour, which is mostly bronzish. Elytra present on both sides single rows of periscutellar punctures, with the first row from the centre being shorter and just from the second to the sixth row being regular in length (Fig. 3).

The head is hypognathous and presents, between antennal sockets, a narrow and convex frontal ridge, with a width ratio compared to antennal socket width of about 1.60. Head also presents a well-defined and relatively deep suprafrontal sulcus. Frons presents relatively long setae on sides. The head vertex is mostly flat, with, on its surface, 8-10 or 3-5 punctures near eye. Antennae are divided into 11 antennomere, most of which are yellow or partially dark brown coloured.

Pronotum is also bronzish in colour, with its sides slightly convex and maximum width near the base, which does not present longitudinal impunctate strip. Contrarywise, the area adjacent to mid-basal margin of pronotum is covered with punctures.

Chaetocnema tibialis tibiae are generally brown, rarely yellow, its femurs are also brown in colour.

Chaetocnema tibialis male's first protarsomere is slightly longer and wider than the second one. First male metatarsomere is also longer than second one, with a ratio of about 1.6, but is slightly less wide. Third male metatarsomere is about 2.5 times longer than the fourth one. Metatibiae present a sharp, large lateral denticle. The total metatibial length compared to the distance between denticle and metatibial apex is about triple.

Since *C. tibialis* is similar to *C. breviuscula*, *C. delarouzeei*, *C. lubischevi*, and *C. scheffleri*, these species can be best recognized by investigating the shape of the aedeagus, the proportions of the body and some differences in punctation of elytra and pronotum. In *C. tibialis*, the aedeagus is generally cylindrical along its length with the apex strongly curved ventrally in lateral view, with a poorly differentiated apical denticle.

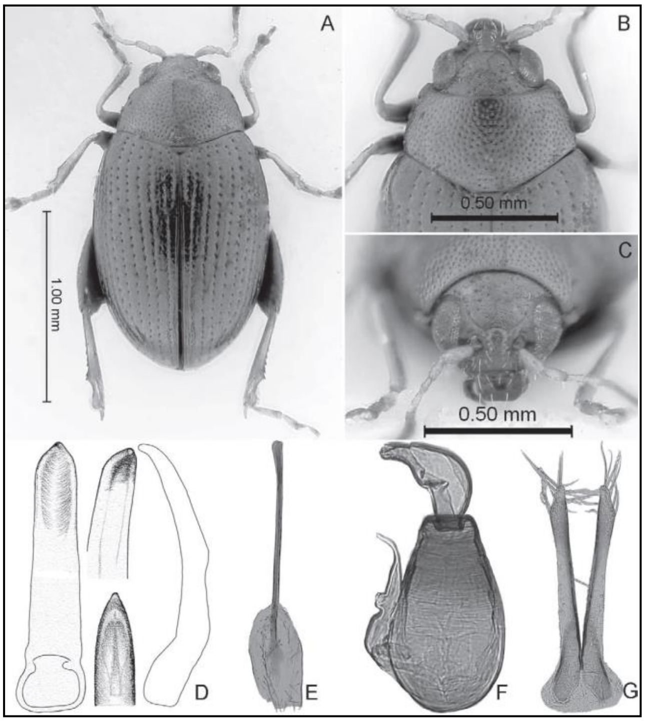

Figure 3 -from Konstantinov et al., 2011. Chaetocnema tibialis; A, habitus, dorsal; B, pronotum, dorsal; C, head, frontal; D, aedeagus, ventral, lateral, and dorsal; E, tignum; F, spermatheca; G, vaginal palpi.

Adults of the genus *Phyllotreta* share several common morphological characters. Their body generally ranges from 1.50 to 3.62 mm, with an elongate oval shape. Female specimens are usually bigger than males. The surface of their dorsal part of the body is glabrous, lightly pubescent just in the ventral part. The elytra surface can be unicolor, generally completely dark with uniformly punctation or can present a median pale stripe or even 1 or 2 pale stripes on each elytron (Fig. 4). The antennae have 11 segments, and their length is about half of the body length. Regarding their legs, they present a narrow and hollowed out hind tibia, close to tarsus insertion. They also present a small spur in the hind tibia, inserted about the middle of the tip of the tibia (Duff, 2018). Among *Phyllotreta* genus, which includes over 300 species worldwide, there are several important species in Europe and also in Italy, e.g., *Phyllotreta cruciferae* Goeze, *Phyllotreta nemorum* L., *Phyllotreta striolata* Fabricius, *Phyllotreta undulata* Kutschera, *Phyllotreta atra* Fabricius, *Phyllotreta nigripes* Fabricius, and *Phyllotreta vittula* Redtenbacher. These species are not specific pests of sugar beets, even though they can still have an economic importance on this crop, depending on the weed infestation and their occurrence on neighbouring crops.

Phyllotreta cruciferae adults (Fig. 4) are about 2 mm long, with an unicolorous shining, blue black elytra. The elytra are also covered with a thick punctation, as well as the pronotum. The antennae are slender, mostly black coloured, with the exception of the first 2-3 antennomeres, which are paler (Chittenden, 1927). It also has the tarsi amber or dark-amber coloured. *Phyllotreta atra* and *nigripes* are similar in dimensions, colour, and shape (Fig. 4). *Phyllotreta atra*'s body is entirely black, except for the second and third antennomeres, which have a yellowish tint, while *P. nigripes* has bright bronze-green, with bluish reflections colour, with black antennae. The dorsal part of its body is characterized by a fine and dense punctuation (Fig. 4).

Phyllotreta striolata adults (Fig. 4) are slightly over 2 mm long (Smith, 1973). Their body is mainly black, with a median yellow stripe on each elytron, incurved apically but never meeting at suture. The antennae are black or brown, with the basal 2-3 antennomeres testaceous.

Phyllotreta nemorum, vittula and undulata are similar to *P. striolata* in dimensions, and shape, but can be distinguished by some key aspects of elytra colouration. In particular, the elytra of *P. nemorum* are mostly black but present yellow stripes, slightly inward curving at base and more prominently at apex (Fig. 4). *Phyllotreta vittula*' s body is, for most, black, shining green or bronze on the pronotum, and with narrow yellow stripes on the elytra (Fig. 4). *Phyllotreta undulata* presents a black body, with yellow longitudinal stripes prominently curved towards the apex of the elytra (Fig. 4).

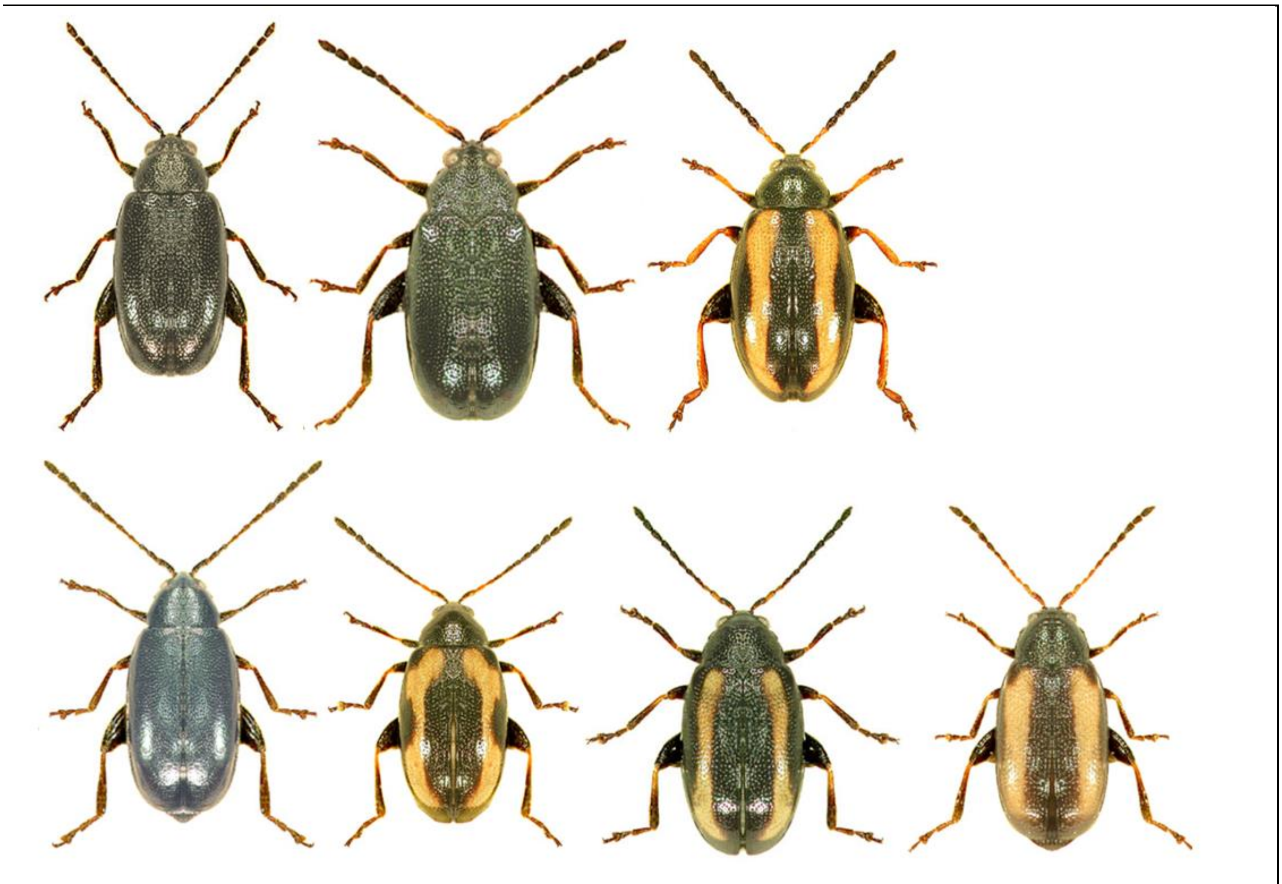

Figure 4 - Upper row, from left: P. atra, cruciferae and nemorum. Lower row, from left: P. nigripes, striolata, undulata and vittula.

Finally, species belonging to genus *Altica* are hardly distinguishable, based on external morphological differences. To ensure the correct identification, the examination of internal genitalia needs to be used, even though molecular analysis represents the most reliable way (Ruhl *et al*., 2010). Indeed, most species of the genus *Altica* share comparable dimensions, an elongate oval shape, and present metallic blue to green elytra, which protect a similarly coloured metallic body that reflect purple and bronze colours (LeSage, 1995). All *Altica spp*. present round eyes and filiform antennae with 11 segments.

1.2.2. Host plants

Chaetocnema tibialis is reported to be a very polyphagous pest, with a wide range of host plants, which include both cultivated and spontaneous crops e.g., *Beta vulgaris* (Bargagli, 1878; Heikertinger, 1925; Jolivet, 1967; Furth 1985), *Spinacia oleracea* (Nonveiller, 1960), and several species of *Salicornia*, *Atriplex*, *Chenopodium*, *Polygonum* and *Amaranthus* (Peyerimhoff, 1915; Nonveiller, 1978; Heikertinger, 1951; Biondi, 1990a; 1990b; Doguet, 1994; Ghadiri, 1990). Moreover, other similar species of *Chaetocnema* genus are reported to feed on plants in the families *Amaranthaceae*, *Asteraceae*, *Brassicaceae*, *Cannabaceae Chenopodiaceae*, *Cyperaceae*, *Fabaceae*, *Juncaceae*, *Poaceae*, *Polygonaceae*, *Rosaceae*, and *Salicaceae* (Augustin *et al*., 1986; Clark *et al*., 2004).

Phyllotreta spp. are oligophagous pests that are mainly attracted by several Brassicaceae species and related plant families in the order Brassicales e.g., Resedaceae, Cleomaceae, Tropaeolaceae (Gikonyo *et al*., 2019).

Many species in the genus *Altica* show a relatively broad host range. One of the preferred family of host plants for *Altica* sp is Onagraceae, which includes popular ornamental plants (Clark *et al*. 2004), but several species are also attracted by Rosaceae, Ericaceae, Corylaceae, Comaceae, and different Brassicaceae species e.g., *Sinapis arvensis*, *Brassica spp., and Raphanus sativus* (Furth, 1980). Weedy plants such as *Epilobium* species may also be host plants for some *Altica* species (Pettis *et al*. 2004).

1.2.3. Biology

In northern Italy, *C. tibialis* overwinters at the adult stage, generally sheltered in the ground at the base of wild plants at the edges of cultivated fields. Subsequently, at the start of the spring period, generally in between March and April (anyway when temperature is above 8-9 °C), they emerge and move to the new fields where sugar beets have been sown and have emerged, to begin their trophic activity. Temperature is one of the most important parameters that drive Chaetocnema species exit from hibernation and starting of feeding activity; 13°C, for different species of the genus represents the optimum (USDA, 1961). Adults continue their feeding activity for about 2-3 weeks after emergence, before becoming sexually matured (Wildermuth, 1917).

Subsequently, the adults mate and lay their eggs into the soil near host plants at a depth of 3-5 cm (Davidyan, 2008). Chaetocnema eggs are generally yellow white in colour, oval in shape, and semitranslucent, very small (about half a mm long). The egg stage can last from 2 weeks to 1 month, mostly depending on temperature.

When eggs hatch, the new-born larvae descend into the ground, without causing economic damage, then they pass through three instars and mature in about a month. Most of *Chaetocnema* larvae are white, with black head, pronotal dorsal plate, legs, and abdominal sclerites (LeSage and Majka, 2010). Full-grown larvae are 4-5 mm long. After maturation, larvae leave the plant in order to pupate into self-constructed earthen cell in the soil.

In northern Italy, these pupae give rise in the summer period to new adults, which can either overwinter directly or, under certain favourable climatic conditions, can originate a second generation. This second generation usually does not cause economic damage on sugar beets because plants are grown enough by that time. Second generation adults feed on the leaves of plants from *Polygonaceae* and *Amaranthaceae* family until middle September, when they migrate to hibernation sites.

Most of the species belonging to the genus *Phyllotreta* share many similar biological aspects. Most species overwinter as adults in a state of reproductive diapause into the soil or in leaf litter near damaged, generally cruciferous, fields (Vig, 1998; Vig, 2003). From overwinter, adults generally emerge in early spring (mid-end march) to start migrating into first crops, which include sugar beet, in order to feed and mate. *Phyllotreta* activity is mainly driven by air temperature. Indeed, they begin being active just when daily maximum temperature reaches 14°C (Lamb, 1983).

Oviposition occurs several centimetres deep in the soil at the base of host plants, generally during the night period. Most of *Phyllotreta* eggs are pale yellow coloured, elongate oval in shape, and about half a mm in length. (Meister, 1969). The duration of the egg stage ranges from few days up to 3 weeks, depending on temperature. (Kinoshita *et al*., 1979). Temperature threshold for egg development is 11.2°C.

The new-born larvae are almost transparent in colour, about a mm long, with large head and anal plates, in proportion to the remaining parts of the body. *Phyllotreta* larvae present three instar stages, during which they grow bigger up until 5-6 mm (third stage larva). Near the end of larval development, larvae stop feeding to begin their pupal stage. The duration of larval stage generally ranges from 3 to 4 weeks.

Phyllotreta pupae are generally white in colour, 2-3 mm long, and exarate. Pupal stage’s duration ranges from around 10 days to 2 weeks.

Therefore, *Phyllotreta* species present an average life cycle from egg to adult emergence that ranges from 1 to 2 month, depending on climatic conditions. Adults then are capable to live up until 40-50 days, with peak of flight activity generally in late April-May.
Phyllotreta can present both univoltine and multivoltine population. Their number of generations per year, indeed, depends essentially on locations. Multivoltine populations of *Phyllotreta* are reported, for example, in India, where *P. cruciferae* can even present 7-11 generations (Bonnemaison, 1965). Contrariwise, in northern Italy, generally, only one generation of the insect occurs annually, with the possibility of a second summer generation, less important.

Altica species also overwinter as adults, inside their pupal cases in the top 1-2 cm of soil or leaf litter. From there, they emerge in early spring to feed on the foliage of their host plant (Lee and Shim, 2003). After a short period, they reach sexual maturation and start mating. Eggs are generally laid on the upper or lower surfaces of the host plants leaves. Eggs are 1-2 mm long, oval in shape and hatch after about a week after oviposition. As soon as eggs hatch, the new-born larvae start feeding on host plants leaves. Many *Altica* species show gregarious behaviour, when feeding. They are generally dark coloured, from brown to black, and are around 5 mm long. They go through three larval instars before pupating into pupal cases constructed, using mucus, into the soil (Pettis *et al*., 2004). *Altica* species may produce one to three generations per year, depending on location (Chappell *et al*., 2012), at northern latitudes, they typically show univoltine generations, but at lower latitudes, there can be two to three generations per year.

1.2.4. Damage

Chaetocnema tibialis damage on sugar beet is determined by the adult stages, which, with their robust chewing mouthparts, carry out small roundish erosions (1 mm in diameter) on the leaf limb. These erosions only affect the mesophyll and the lower epidermis. Generally, indeed, the upper epidermis is left undamaged, but, subsequently, it necrotizes and detaches as the leaf grows, creating a peening of the leaf, which is perforated in several points, decreasing photosynthesis capacity. Sometimes *C. tibialis* feed on the stem in addition to the leaves (Bažok, 2006).

If the attack is precocious and affects the young seedlings, with plants at cotyledonary stage, serious failures can occur, due to the death of the seedlings themselves. Indeed, when the plant is at the cotyledon stage, one flea can cause 33% damage per day, three fleas up to 62% and five fleas can cause as much as 90% damage to the plant (Maceljski, 2002). Sugar beets most susceptible period for flea beetle damage ranges from cotyledon stage to 6-8 true leaves stage; subsequently, the plant's capacity to well tolerate attacks is higher.

Contrariwise, the larvae, which live in the ground, in the rhizosphere, are not considered harmful to sugar beet.

Similarly, other flea beetle species of the genera *Phyllotreta* and *Altica* can cause direct damage to sugar beet, which is dealt by adult stages. Adults generally gnaw small pits or holes on the upper epidermis and parenchyma of the leaves. Those, during heavy infestations, can merge to form larger holes on the leaves, first reducing plant's photosynthetic capacity. Later damaged leaves may wilt, leading to severe delay in plant growth and yield reduction (Popov, 1958). Larvae, instead, can occasionally injure roots or mine leaves of other host plants; indeed, they usually are not harmful to sugar beet.

Moreover, flea beetle larvae and adults are also able to cause indirect damage to plants by the transmission of pathogens from infected cruciferous plants to healthy ones during feeding (Dillard *et al*., 1998; Saharan *et al*., 2005; Shelton and Hunter, 1985; Stobbs *et al*., 1998).

1.2.5. Control measures

In past years, management of flea beetle infestations was generally carried out with seed treatments, based on neonicotinoid (mainly imidacloprid and thiamethoxam) coatings of the seeds. These treatments have shown good efficacy in controlling not only flea beetles early infestations, but also those of wireworms and sugar beet weevils at low population (Viric Gasparic, 2021). However, recently, following the European neonicotinoid ban, the use of the active substances imidacloprid, clothianidin and thiamethoxam in the field has been completely prohibited because of the risk to bees. Therefore, sugar beet production remained without an important defence weapon, while pest pressure is rapidly increasing. For this reason, more and more attention is being paid to the study of effective control alternatives that allow to manage these dangerous pests for the time necessary so that sugar beets are no longer susceptible to their attacks.

Regarding flea beetles, first step to correctly manage their infestations surely is monitoring. It should be carried out as soon as sugar beets start to emerge. It can be realized both by visual and traps monitoring. Visual monitoring should be aimed at the assessment of both the number of flea beetles infesting plants and the number of holes per leaf caused by pest's feeding activity, in representative areas of the field. Traps monitoring, instead, can be carried out by placing some chrome-attractant yellow glue traps among sugar beet plants and by counting the total number of flea beetles captured from cotyledon stage to 6-8 true leaf stage.

Following the Integrated Production Regulation issued by Emilia-Romagna region (northern Italy), damage threshold, above which it is advisable to carry out a specific insecticide treatment, are set as follows:

- presence of any holes on the cotyledons.
- presence of 2 holes/leaf on plants with 2 true leaves.
- presence of 4 holes/leaf on plants with 4 true leaves.

Exceeded these threshold, insecticidal treatments are carried out using permitted insecticides, mainly based on etofenprox, lambda cyhalothrin, cypermethrin, and deltamethrin. However, the use of chemical insecticides to control these pests should be limited as much as possible, because of its hazardous effects on the environment and because some chemical substances could residue into leaves or pulp.

Moreover, those chemical insecticides could only be used in in integrated or conventional farming, but from an organic farming perspective, those are obviously not permitted.

For this reason, non-chemical methods to manage flea beetle infestations are required in organic farming.

Noteworthy agronomic practices that can contribute to reduce flea beetles damage on sugar beet seedlings (excluding trap-cropping technique, which will be deepened in chapter 1.3), are those which enhance a fast germination of the seed and promote seedling growth, such as timely sowing and the use of healthy and large seed for planting (Elliott *et al*., 2008; Milbrath *et al*., 1995). Also conservative tillage can show a reducing effect on the pressure of flea beetles infestations (Dosdall *et al*., 1999).

In literature, some examples of natural enemies are also reported, both of flea beetle's adult forms and larval forms. The main natural enemies of flea beetles are hymenopterous wasps (Ulber and Williams, 2003), such as the braconids *Microctonus spp.* and *Townesilitus spp*., but their contribution to the management of flea beetle infestations is limited (Knodel and Olson, 2002).

A restricted level of predation by some generalist predators, such as lacewing larvae (*Chrysoperla spp*.), and damsel bugs (*Nabis spp*.) has also been reported (Knodel and Olson, 2002).

Moreover, overwintering flea beetles and larvae in the soil are susceptible to soil-dwelling entomopathogenic nematodes belonging to *Steinernematidae* and *Heterorhabditidae* families (Ambrosino, 2008).

Furthermore, the entomopathogenic fungal species *Beauveria bassiana* (Bals. - Criv.) Vuill. and *Metarhizium brunneum* Petch have shown a good efficacy in reducing flea beetle damage and population density (Reddy *et al*., 2014).

Finally, a biological control agents of *C. tibialis* have also been reported. Belonging to the group Microsporidia, which includes some of the most important pathogens of insects, *Nosema chaetocnemae* (Microspora) represents the first parasitic microsporidid identified from *C. tibialis* (Yaman and Radek, 2003). *Nosema* genus counts several species, parasites of different insect pests (Handel *et al*., 2003; Hokkanen and Lipa, 1995; Lipa and Hokkanen, 1992), but *N. chaetocnemae* represents the one specific for *C. tibialis*; it is, therefore, of a potentially great importance and interest in the future management approach to this pest, from an organic farming perspective.

1.3. Trap-cropping technique

Trap cropping is an agronomic practice, which can be well implemented in an agroecological set of practices, as it fits into the ecological framework of habitat manipulation of an agroecosystem for the purpose of pest management (Shelton and Badenes-Perez, 2006). One of the most witnessed definitions of trap cops is: “plant stands grown to attract insects or other organisms like nematodes to protect target crops from pest attack, preventing the pests from reaching the crop or concentrating them in a certain part of the field where they can be economically destroyed” (Hokkanen, 1991). This definition, which implies that pests always show a differential preference between the plant used as a trap crop and the plant to be protected (cash crop), has been questioned for its limited view. This definition, indeed, places a lot of emphasis on the key concept of differential preference. In some trap cropping systems, however, this difference in terms of pest preferences does not occur as, for example, it is the same species of plant to be protected that is utilized as the trap crop, if grown in a particular spatial or temporal manner.

A broader definition of trap cropping has thus been proposed by Shelton and Badenes-Perez in 2006. They defined trap crops as “plant stands that are, per se or via manipulation, deployed to attract, divert, intercept, and/or retain targeted insects or the pathogens they vector in order to reduce damage to the main crop”.

Therefore, trap crops aim to protect the so-called cash crop mainly by materially preventing pests from reaching the crop or by making them gather where they can be managed without causing damage to the protected crop (Landis *et al*., 2000). This aim can be achieved through time or spatial manipulation of plants in order to offer host plants to concentrate pests in a desired site with the correct timing.

In organic farming, where pest management is carried out mainly through habitat manipulation or other biological control practices (Zehnder *et al*., 2007; Klopatek and Gardner, 1999), trap cropping permits the enhancement of biological control, offering greater host plants diversity for natural enemies while simultaneously complicating the pest habitat. Indeed, trap cropping represent an example of crop species polyculture, which often leads to a pest damage reduction compared to monocultures within a given area (Andow, 1991; Letourneau *et al*., 2011).

There are some key aspects in a trap cropping design to be successful. Firstly, it is important to correctly match the volatile compounds released by the trap crop species with the target pest to control; indeed, different trap crop species can release different volatile compounds, capable to attract or repel different pests and natural enemies (Dicke and Hilker, 2003; Reddy, 2002; Zhu *et al*., 1999). In this regard, since a multi-compound blend has proved to be more attractive than a single chemical constituent, the pairing of different trap crop species can provide longer attraction effects on insect pests. Another important aspect for a trap crop to be effective is the similarity in terms of agronomical requirements, mainly light and temperature demands, with the crop to be protected. The spatial-temporal deployment of the trap crop around the cash crop is also a fundamental aspect to be taken into consideration for its effectiveness.

There are indeed many modalities of trap cropping, classified depending on plant characteristics or on plants deployment.

Based on trap crop characteristic:

- Conventional trap cropping: it is defined as the general practice in which, next to a high value crop, a more attractive trap crop species is grown in order to offer to pests a preferred food source or oviposition site, thus reducing the arrival or the damage to the main crop (Javaid and Joshi, 1995).
- Dead-end trap cropping: it is a trap crop modality, in which plants used are very attractive to pests, but on which they or their offspring is not capable of surviving (Shelton and Nault, 2004). These plants are, in this way, used as a sink by pest in early season but avoiding their future movement from trap crop to cash crop (Badenes-Perez *et al*., 2004). The dead-end effect of trap cropping can also be achieved by treating them with conventional insecticides.
- Genetically engineered trap cropping: this technique consists in the planting of genetically modified plants, which still needs to be more attractive to the targeted insect pest, around the main crop. An example of this trap cropping methodology is the early plantings of Bt potato (potato plants which have been genetically engineered to express proteins from *Bacillus thuringiensis*) to trap *Leptinotarsa decemlineata* populationson later cultivated potatoes.

Based on deployment of the trap cropping:

- Perimeter trap cropping: this term refers to trap crop plants that are sown or planted around the borders of the cash crop (Boucher *et al*., 2003), generally surrounding the edges of the field. This practice is the most used in common IPM pest control strategies based on trap cropping. It is a very useful control strategy for pests which moves to fields from overwintering sites next to the crop (Potting *et al*., 2005).
- Sequential trap cropping: it indicates trap crops that are sown/planted earlier and/or later compared to the main crop, in order to express trap crop’s attractiveness towards the desired pests, luring their population away from the cash crop for the most delicate and serious moments.

- Multiple trap cropping: this term refers to the planting of different trap crop species at the same time, aiming at either managing different pests simultaneously or at increasing the control of only one pest combining plants attractive to the pest at different stages.
- Push-Pull trap cropping: this trap cropping modality will be further deepened in Chapter 2.3.

Some other trap cropping modalities are reported e.g., biological control-assisted and semiochemicals assisted trap cropping, and can provide important contributions to pest control.
Trap cropping design and deployment need to be specific for each target pest; therefore, knowledge of pests behaviour is necessary in planning a trap cropping design.
The required size of the trap crop is also a function of the pest species and of its expected populations and mobility, but, generally, the proportion of the trap crop ranges from 10 to a maximum 20 % of the main crop area.

Regardless of trap cropping methodology implemented, the technique offers several advantages. These include both economic and environmental benefits such as: the reduction in pest damage on the cash crop (if the strategy is successfully applied), the lesser need to apply specific insecticidal treatments, the improvement in crop's quality, the better soil and environment conservation, the higher production, the enhancement of biodiversity, and the conservation of natural enemies. Generally, indeed, the savings resulted from lower pest damage and insecticide use can outweigh the costs of trap crops maintenance.
Despite the advantages offered from using trap crops, there are also several concerns. Among these: the need for knowledge of pests behaviours, the need for additional agronomic planning and additional used resources. Moreover, if trap cropping is not successful, can lead to the creation of “pest nurseries,” which can lead to a more rapid or widespread pest outbreak.
Despite the disadvantages, when trap cropping technique is correctly implemented, it has great potential to keep pests below economic damage threshold and to be employed as pest management practice, especially in organic farming (Kachhawa, 2020).

1.3.1. Successful examples of application

Since the 1930s, several successful trap cropping examples have been reported for the management of various insect pests. Of these, only few successful cases, which targeted mainly Coleoptera, Hemiptera and Lepidoptera species, have highlighted an importance from a commercial standpoint. All of these trap cropping systems were meant for pests that aggregate and moved on attractive plants. Indeed, for a trap crop to be effective, it is necessary to correctly determine the insect stage targeted/attracted, the insect’s capacity to aggregate and direct its movement and the insect’s modality of colonization. In particular, among these, the insect stage targeted from trap crop is of critical importance in designing an effective trap crop strategy. Indeed, for Lepidoptera, trap crop species selection requires knowledge of the ovipositional preference (since the adult select plants for oviposition, but are the larvae that cause damage), while, in the case of flea beetles, since it is the adult stage the one causing damage, knowledge of adult feeding preference is required.

Among most successful examples of trap cropping systems, the following can be reported:

- The use of squash (*Cucurbita spp*.) in sequential or semiochemically assisted trap cropping designs to target the striped cucumber beetle (*Acalymma vittatum* Fabricius) in the United States.
- The use of Chinese cabbage (*Brassica rapa* subsp. *pekinensis*), marigolds (*Tagetes spp.*), oilseed rape (*Brassica napus*), and sunflower (*Helianthus annuus*) in multiple trap cropping design to target the pollen beetle (*Meligethes aeneus* Fabricius) on Cauliflower in Finland.
- The use of squash (*Cucurbita spp*.) in sequential or semiochemically assisted trap cropping designs to target the squash bug (*Anasa tristis* De Geer) on watermelon in the United States.
- The use of alfa-alfa (*Medicago sativa*) in conventional trap cropping design to target *Lygus* bugs (*Lygus lineolaris* Palisot de Beauvois and *Lygus rugulipennis* Poppius) on cotton and lettuce respectively, in the United States and Sweden.
- The use of genetically engineered Papaya (*Carica papaya*) to target Papaya aphids (*Myzus periscae* Sulzei, *Aphis gossypii* Glover and *Aphis craccivora* Koch) in the United States.
- The use of Napier (*Pennisetum purpureum*) and Sudan grass (*Sorghum drummondii*) in push-pull trap cropping design to target the spotted stem borer (*Chilo partellus* Swinhoe) on maize and sorghum in several African regions.
- The use of Indian Mustard (*Brassica juncea*) in sequential or conventional trap cropping designs to target the diamond moth (*Plutella xylostella* L.) on cabbage in several states, including Sweden, India, United States and South Africa.

In addition to these, which are the most successful examples of trap cropping, there are many other cases that have shown positive results on a field scale, but that have not yet been implemented at the level of economic importance. For example, good field results have been highlighted from the use of Soybean and mustard to target the stink bug complex (*Euschistus heros* Fabricius, *Nezara viridula* L., and *Piezodorus guildinii* Westwood) in sequential or conventional trap cropping designs on soybean and maize respectively, in Brazil, Nigeria and New Zealand. Another interesting example of a successful conventional trap cropping design is the use of squash and cucumber to target whiteflies (*Bemisia tabaci* Gennadius and *Bemisia argentifolii* Bellows-Perring) on tomato and bean respectively, in Lebanon and United States.

1.3.2. Attractive plants for flea beetles

Many flea beetle species express a distinct preference for specific host plant, with many of them included in the family of Brassicaceae (Nielsen, 1988; Aslan and Gök, 2006).

Indeed, especially crucifer flea beetles, are highly attracted to *Brassica* plants for the chemical components of glucosinolates, whose chemical profiles vary between different brassica species. In particular, allyl isothiocyanate, which is a breakdown product of glucosinolates in *Brassica* plants, is one of the most attractive component for adults of many *Phyllotreta* species (Feeny, *et al*., 1970).

Among Brassicaceae family, a study conducted to find the most attractive plants to *Phyllotreta spp*. highlighted that adults of that genus preferred, in decreasing order (Metspalu *et al*., 2014):

- *Eruca sativa*,
- *Brassica juncea*,
- *Brassica nigra*,
- *Raphanus sativus*,
- *Sinapis alba*,
- *Brassica rapa*,
- *Brassica napus*
- *Camelina sativa*.

Other studies showed that also *Barbarea vulgaris* and *Sinapis arvensis* could be used as trap crops in order to manage *Phyllotreta cruciferae* (Root and Tahvanainen, 1969, Altieri and Gliessman, 1983, Altieri and Schmidt, 1986), *B. vulgaris*, in particular, also showed resistance to *P. nemorum* and *crucifere* attacks, because of its saponin content (Agerbirk *et al*., 2003; Kuzina *et al*. 2011; Christensen *et al*. 2014).

A further survey recommended the use of a multiple trap cropping design based of *B. napus*, *R. sativus, and S. alba* (Bohinc and Trdan, 2013), since the attraction of plants to *Phyllotreta spp.* can vary during the course of the season (Badenes-Pérez *et al*. 2017). Another research also concluded that a mixture of different trap crop species, containing *Brassica juncea*, *B. napus*, and *B. campestris* var. *chinensis* better protected the cash crop from flea beetle attacks than the same plants used as trap crops alone (Parker, 2012). Indeed, combining different plant species, with different phenologies, physical structures and chemical profiles can surely increase the trap crop attraction to different flea beetle species.

2. *EVALUATION OF THE EFFECTIVENESS OF A SEMIOCHEMICAL-ASSISTED TRAP CROPPING STRATEGY AGAINST EUROPEAN TARNISHED PLANT BUG (Lygus rugulipennis) ON LETTUCE*

2.1. Economic, Descriptive and Market Data on The Production of Lettuce

Lettuce (*Lactuca sativa* L.) belongs to the botanical family of Asteraceae (Compositae), which is thought to be the largest of all plant's families, comprising more than 23,000 species (Bayer, 1998; Bremer, 1994; Funk *et al*., 2005). Lettuce as a plant is native to southwest Asia (De Vries 1997; Zohary *et al*., 2012), where the wild lettuce species *Lactuca serriola* was present. From there, cultivated lettuce was domesticated in the Mediterranean area (Durst 1930; Harlan 1992). There are several cultivars of lettuce, which differ in shape, structure, chemical composition, qualities, adaptation to climatic condition, hardiness, and yield (Leon *et al*., 2012).

There are seven main cultivar groups of lettuce, each including many varieties (Trehane, 1995):

- Leaf (or cutting): this lettuce cultivar has loosely bunched leaves, which are usually consumed fresh. This group is usually referred to as either green leaf or red leaf, based on leaf colour, which ranges from yellowish green to various shades of red, mainly depending on anthocyanin content and light intensity during growth (Still, 2007). This is the most widely planted among lettuce cultivars.
- Iceberg (or Crisphead): this cultivar is very heat sensitive. Indeed, it was originally cultivated in cold areas of United States, from where it was carried on trains whose wagons were filled with crushed ice, on top of which lettuce heads appeared as icebergs. This cultivar is very low in nutritional power, mainly due to the high presence of water and fibres (around 90% of the total weight).
- Butterhead: this cultivar is also known as Boston lettuce, and is characterized by forming loose, green, or even reddish, heads. It also shows a characteristic sweet flavour and a tender texture.

- Romaine (or Cos): this is a lettuce cultivar that develops a long head of robust dark green leaves, characterized by firm ribs in their centres. Unlike most lettuce cultivars, it is more tolerant to heat.
- Summercrisp: this lettuce is midway between Iceberg and Leaf cultivars, but, compared to these, tends to be larger, more bolt-resistant and better flavoured.
- Celtuce (or Stem): this cultivar is primarily grown for its seedstalk, rather than for its leaves. It is largely used in Asian cooking, primarily in Chinese cuisine.
- Oilseed: this cultivar is grown for its seeds. It its characterized by the presence of few leaves, by a quick bolting and by the production of seeds that are around 50 percent larger than other types of lettuce. The seed are then pressed to extract an oil mainly used for cooking.

Leaf, Romaine, and Iceberg are the most cultivated and consumed cultivars worldwide.

Originally, Europe and North America were the market leader for lettuce, but, by the late 20th century, the consumption of lettuce had spread throughout the world. Nowadays, indeed, lettuce is grown in 106 countries on 1,226,370 harvested hectares for a total production of 27,660,187 tonnes (FAO, 2022).

Of the global lettuce production, Asia contributes for a 63 %, with 17,427,652 tonnes produced, followed by Americas (20.3 %, 5,609,872 tonnes), Europe (14.1 %, 3,892,163 tonnes), Africa (2.1 %, 567,583 tonnes), and also Oceania (0.7 %, 162,917 tonnes) (FAO, 2022).

In 2020, China was the biggest grower of lettuce, with a harvested area of 606,194 hectares, which is nearly half (49.43%) of the global hectares in which the crop is grown, also exceeding half of the global lettuce production, with 14,318,667 tonnes.

The other top lettuce producers are United States (with 4,402,375 tonnes), India (with 1,121,379 tonnes), Spain (with 969,060 tonnes), Italy (with 735,470 tonnes), Japan (with 580,546 tonnes), Mexico (with 541,804 tonnes), Belgium (with 538,900 tonnes), Turkey (with 520,131 tonnes), and France (with 516,880 tonnes) (Fig. 5).

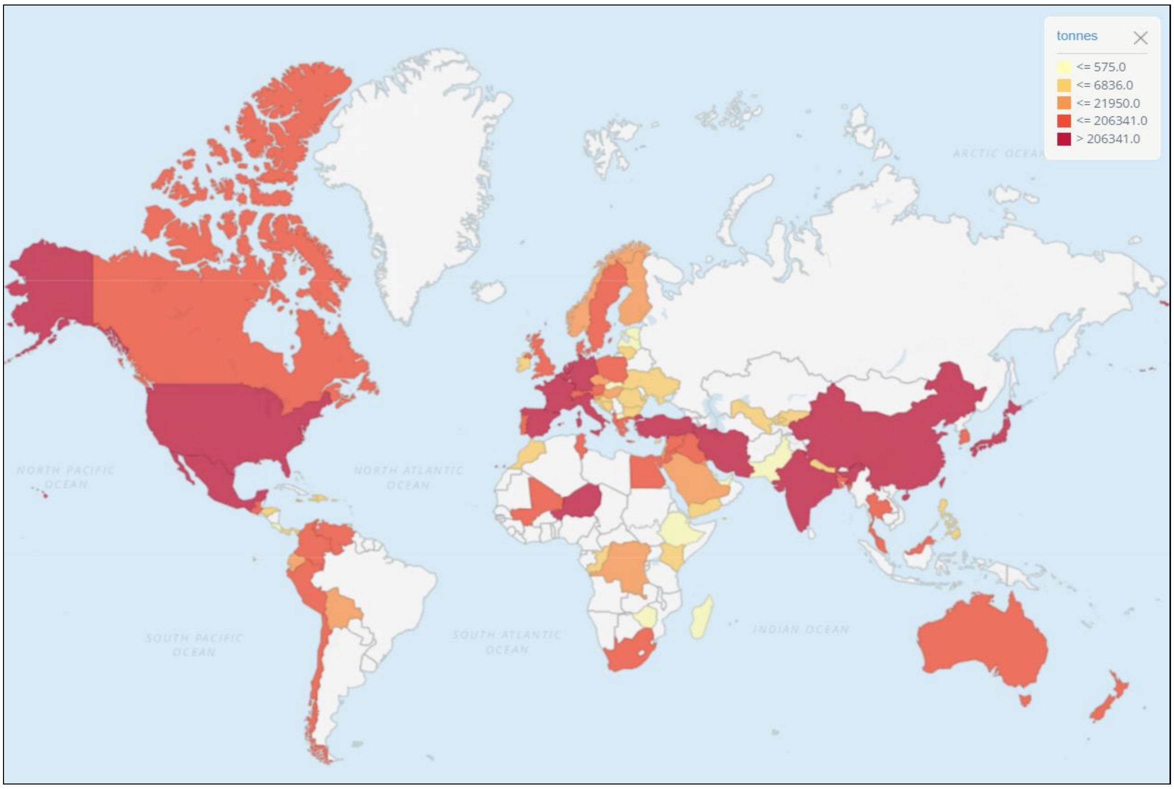

Figure 5 - Global lettuce production by country (Faostat, 2022).

In Italian market, over the last decade, a slight decrease in the area destined for lettuce cultivation, as well as its production, has been observed (Fig. 6).

In Italy, lettuce is nowadays harvested on 32,100 hectares, for a total production of 735,470 tonnes (year 2020) (FAO, 2022).

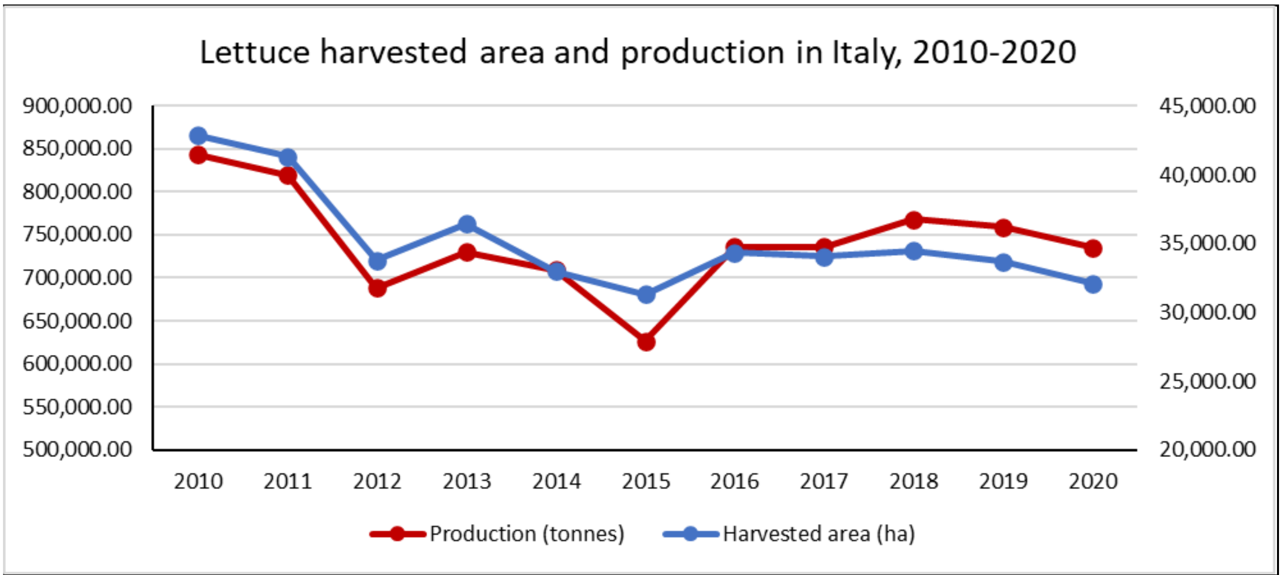

Figure 6 -Italian lettuce harvested area and production (Faostat, 2022).

2.1.1. Insect pests and diseases

Lettuce is one of the most demanded salad crops in both fresh and ready-to-use markets around the globe (Fallovo *et al*., 2009), that can be grown throughout all year, under different climatic conditions, by choosing the most suitable cultivars. Unfortunately, during the year it can be exposed to several fungal diseases, insect or nematodes attacks, and viruses.

Many insects are attracted to lettuce, and some of them are able to severely threat its cultivation, including aphids, armyworms, mirid bugs, lettuce leaf miner, thrips, and whiteflies. Among these, aphids are the most common found damaging lettuce. Among the various species of aphids, the green peach aphid (*Myzus persicae* Sulzer), the potato aphid (*Macrosiphum euphorbiae* Thomas) and the lettuce aphid (*Nasonovia ribisnigri* Mosley) are those of major importance (Reinink and Dieleman, 1993; Parker *et al*., 2002). Among these species, *N. ribisnigri* is surely the most difficult to manage, as is very widespread throughout the entire lettuce growing season but mainly due to its feeding behaviour. This aphid, indeed, forms large colonies inside the lettuce head, being therefore protected from foliar insecticide applications, where it feeds on the heart leaves, making lettuce unmarketable (Mackenzie and Vernon, 1988). Since they show lower infestations during the growing season, the other two aphids species, instead, are considered less dangerous, also considering that their feeding preference is oriented on outer leaves only (Shrestha *et al*., 2017). In general, aphids direct damage on lettuce results in leaf distortions, decreased seedling vigour. Severe aphids infestations can change leaf colour and bring to lettuce heads deformation, which leads to reduced crop yield. (Fletcher *et al*., 2005). Lettuce aphid is also capable of transmitting virus diseases, including LMV (Blua, 1997). Aphids on lettuce are generally controlled by using permitted insecticidal treatments but, if on one hand on *M. persicae* and *M. euphorbiae* they usually effectively reduce infestation levels, on the other hand on *N. ribisnigri* it is practically impossible to effectively strike with foliar applied treatments during head maturation, since this aphid's propensity for colonizing the young leaves (Liu, 2004). Moreover, most aphids have also demonstrated to rapidly develop resistance against several insecticides (Barber *et al*., 1999; Stufkens and Wallace, 2004). In nature there are several natural enemies of lettuce aphids, such as predators, (including syrphids and lacewings), and parasitoids. Those natural enemies presence could be enhanced through the implementation in lettuce fields of insectary plants. Additionally, the selection of lettuce cultivars resistant to lettuce aphid is one of the safest and most widely used practice.

Among lettuce arthropod pests, armyworms are also very dangerous. In particular, the most common armyworm species found damaging lettuce are *Autographa gamma* L., *Helicoverpa amigera* Hübner, and *Spodoptera exigua* Hübner, all belonging to the family Noctuidae. Adults of those species are similar in dimensions (between 25 and 45 millimetres of wingspan), but differ in colour, with *S. exigua* forewings being greyish ochreous, *H. armigera* yellowish to orange in females and greenish grey in males, and *A. gamma* brown and grey, with a silver-coloured mark shaped like the Greek letter Gamma in the centre of each forewing. Adults of these species don't represent the harmful stage as they feed on flowers (nectar). On lettuce, indeed, damage is carried out by the larvae, which feed on leaves, starting from the edge towards the midrib, consuming the leaves completely or sometimes leaving pieces of the midrib. Newly hatched larvae start feeding on the section of leaf where their eggs hatch. Subsequently, they hide down under the head-covering leaves and start feeding, often avoiding detection and treatment. Larvae continue their feeding activity on developing heads until maturity, when they then leave the plant to enter the soil, where they pupate. These species are able to make multiple generations, up to 4 for A. gamma, up to 6 for S. exigua and H. armigera (Wilson, 1934; Imura *et al*., 2002). Since these pests ability to hide inside the lettuce heads and their rapid insecticide resistance development, the use of chemical insecticide is usually not recommended. Fortunately, however, there are numerous natural enemies that are able to control these pests. Among them, several parasitoids species, belonging both to Hymenoptera family of Braconidae and the Diptera family of Tachinidae are present. Moreover, there are some predators that frequently attack the both the eggs and the newly hatched larvae, such as *Orius spp*. and *Nabis spp*. Furthermore, on the market there are pheromone traps that can be used to detect the presence of adults, and to disrupt mating in order to limit reproductions.

Another economically important arthropod pest of lettuce is the leaf-miner *Liriomyza huidobrensis* Blanchard. It is native to Central and South America and was first detected in Europe at the end of 1980s in Netherlands, but it has since spread throughout all Europe, especially the Mediterranean region. It is highly polyphagous pest, able to feed on over 15 plant families. The adult has a small (1.3-2.3 mm), greyish-black body, with a bright yellow central area of the scutellum, of the head, and pleura, while the larvae are maggots up to 3.25 mm long, yellow orange in colour. Adult females pierce lettuce leaves, causing wounds that are used for feeding and oviposition site (Mujica and Cisneros, 1997). Eggs are posed under leaf surface, in variable number, mainly depending on the temperature. Eggs hatch in 2-5 days and newly hatched larvae begin their leaf miner activity, usually creating white mines. The damaged is also characterized by the presence of black wet or dried brown areas near leaf midrib or lateral veins. *Liriomyza huidobrensis* mines are characterized by a serpentine irregular shape. If those damage occur on young plants, they can be severely harmed and even die, whereas, on older plants, reduced photosynthetic activity caused by the mines leads to lower yields. On lettuce, even few mines on leaves can make the crop unmarketable. Major control measures against this leaf miner rely on chemical insecticidal treatments and on biological control.

Thrips are also very dangerous pests of lettuce. These insects, which belong to the order Thysanoptera, and to the family Thripidae, are worldwide distributed, depending on species. The two main species found damaging lettuce are *Thrips tabaci* Lindeman and *Frankliniella occidentalis* Pergande. Identifying those two species can be very difficult, as they are quite similar from a morphological point of view, with *F. occidentalis* being about 1.5 mm long and generally light yellow in colour, while *T. tabaci* is slightly smaller (only 1.2 mm long) and yellow coloured, with brown blotches on the thorax and abdominal terga. Reproduction modality, instead, can be different between the two thrips species, being asexual in *T. tabaci*, with unfertilized females giving birth only to females (parthenogenesis), while it can be sexual or asexual in *F. occidentalis*, with unfertilized females giving birth only to males while those which are fertilized give birth to females. Usually, thrips are present throughout the entire lettuce season, but are most abundant after temperature increase, starting from the end of the spring. Thrips can colonize weedy areas next to crop fields, from where they move into lettuce in large numbers when host plants begin to dry down. Thrips are dangerous on lettuce for both direct and indirect damage. Direct damage is made by thrips sucking cells contents in the epidermis of lettuce leaves. Symptoms on leaves appear as small irregular lesions, with metallic reflections, that gradually necrotize. Affected leaves tend to chlorate and take on a dull colour. Indirect damage is also very dangerous as thrips can be vectors of several virus disease, including Tomato spotted wilt virus (TSWV), which can lead to lettuce wilting. Management of thrips infestations starts with the identification of the species, through a monitoring carried out using blue coloured glue traps. In order to limit thrips infestation on lettuce it is important to eliminate weeds next to lettuce field. In case of confirmed infestations, the application of permitted insecticide is recommended, even though resistance to insecticides is known in these pests.

Finally, lettuce can also be severely harmed by, root knot nematodes (*Meloidogyne spp*.), snails (*Helix spp*.), slugs (*Limax spp*.) and several mammals, including rabbits and groundhogs.

Among fungal diseases, the most dangerous for lettuce are the bottom rot, the downy mildew, the lettuce leaf drop, the grey mold and the powdery mildew.
Lettuce bottom rot is a disease caused by *Rhizoctonia solani* Cooke, which is one of the most common soil-borne plant pathogens, found wherever lettuce is grown. The pathogen infects lettuce over a wide range of temperatures but is favoured by warm (20 to 25 ºC) and high-moisture conditions. The pathogen is capable of surviving for long time in soil, colonizing the organic matter, and can be carried for long distances on infected plant parts. The pathogen sclerotia can germinate in damp soil and, when environmental conditions favour disease development, can enter into lettuce through wounds or through stomata on bottom leaves touching the soil. Symptoms firstly appear on those bottom leaves and appear as small, sunken, reddish-brown lesions primarily on the underside of leaf midribs, covered by the white to brownish mycelium. The pathogen can then spread upward from leaf to leaf until the entire head is colonized. Since the pathology is caused by a soil-borne fungus, a common management practice to control the disease is correct crop rotation, thus avoiding planting lettuce immediately after other crops known to be susceptible to *R. solani*. Other control strategies are often based on the use of permitted fungicidal treatments.

Another important lettuce disease is the downy mildew, which is caused by the obligate parasitic fungus *Bremia lactucae* Regel, Bot. Ztg. This disease occurs more often in cooler and wetter growing regions, as low temperatures and a high moisture on the leaf surface is fundamental for spore germination and infection. Therefore, downy mildew infections are more common in early spring or late fall growing periods. When the leaf surface is wet, this pathogen's spores are able to germinate and to penetrate into epidermal cells. From there, the pathogen establishes into leaf tissue, where it produces abundant sporangia, which then emerge through stomates, and are released into the air thanks to the wind, to cause new infections. On infected plants, first symptoms appear as light-yellow angular patches on the upper side of leaves, followed by a white fluffy growth, containing pathogen's spores, on the corresponding part of the lower leaf surface. The infected areas are limited by leaf veins. On red lettuce cultivars the initial spots may appear more greyish, and water soaked. First leaves to show these symptoms are often the older and closer to the ground. Downy mildew can sometimes reduce both the yield and the quality of the crop, as infected older leaves can be easily removed at harvest, but infections on the younger leaves may result in leaving heads in the field. Moreover, leaf tissue damaged by downy mildew can also be an access site for secondary rot pathogens.

The most effective management practice of downy mildew is the use of resistant or tolerant cultivars of lettuce, even though to choose the right variety to grow, the knowledge of the present strain(s) of the pathogen is needed. From an agronomical standpoint, the use of a drip irrigation system as opposed to a sprinkler system, in order to reduce leaf wetness, can help to avoid favourable conditions for pathogen's infection. Moreover, the application of fungicides, focused when environmental conditions are favourable to pathogen, can effectively suppress disease development.

Leaf drop of lettuce can be caused by two pathogenic fungi, *Sclerotinia minor* Jagger and *S. sclerotiorum* de Bary. These fungi are widespread in almost all areas where lettuce is grown but are favoured by cool (optimum of 15-20°C) and moist conditions. *S. minor* and *S. sclerotiorum* are fungi that can survive into the soil for a long period of time in the form of sclerotia. Under favourable climatic conditions, these sclerotia germinate, in order to infect lettuce both directly through senescent lower leaves and through root tissue near the soil surface. During wet weather, sclerotia can also germinate directly on the soil surface and produce structures called apothecia, which then release ascospores that will be carried by air currents to be deposited on healthy lettuce plants, which subsequently will become infected. Generally, *S. minor* infects the stem and the leaves which are in contact with the wet soil, while *S. sclerotiorum* can also infect any of the upper leaves. Symptoms on infected plants are very similar, appearing as a soft, dark brownish, watery decay. If the stem is infected, a rapid wilt is caused, bringing to the collapse and death of infected plants, usually near harvest period. Moreover, the growth white fungal masses occur on the surface of rotted tissue, which can be used to identify the species of the pathogen. *S. minor* generally produces small sclerotia (0.15-0.30 cm in diameter), whereas sclerotia produced by *S. sclerotiorum* are usually larger (from 0.6 to 1 cm in diameter). Several management practices to control lettuce leaf drop are present and can be implemented. Firstly, it is important to avoid excessively wet soils, avoid excessive irrigations and keep the field surface as levelled as possible to exclude stagnant water. Deep ploughing can also be helpful, as it can bury sclerotia, reducing their ability to germinate and cause infection. Finally, effective disease control can be achieved through the use of promptly timed fungicide applications.

The lettuce grey mold, also known as the lettuce crown rot, is a fungal disease caused by *Botrytis cinerea* Persoon, which is a pathogen of many plant species. *B. cinerea* is an opportunistic pathogen, capable to invade and survive on dead or decaying plant tissue but also in the absence of a living host. It is favoured by cool temperatures, high humidity, and free water on plant surfaces. Other lettuce pathologies, as downy mildew, can also make tissues more susceptible to grey mold. On infected plants, first symptoms appear as dark, brownish-grey water-soaked lesions that occur on oldest, and often already damaged, leaves in contact with the soil. From those leaves, the pathogen rapidly expands through healthy plant tissues, subsequently causing a decay of the crown. Over diseased areas characteristic grey, powdery spore masses form, especially on shaded leaves, which are protected from drying. Usually, lettuce grey mold is considered as a minor disease but if field conditions are favourable can cause significant damage. Cultural control of the disease is mainly based on a good field sanitation, since the pathogen can live on decayed organic debris. Therefore, it is important to correctly remove all residue of previous crops from fields. Moreover, it is important to avoid overhead irrigation, so that plant leaves do not remain wet, preferring drip irrigation. If cultural control isn't able to effectively prevent the disease, several fungicides effective against grey mold on a variety of hosts can be used.

Powdery mildew is one of the most common diseases of lettuce and occurs everywhere lettuce is grown, caused by the obligate parasitic fungus, *Erysiphe cichoracearum* de Candolle. The pathogen grows on the epidermis of leaves, and it is favoured by warm and relatively dry conditions, even though relative humidity of 85% or higher is required for disease development. Under the right conditions, the pathogen is able to rapidly produce on the leaf surface numerous spores, which are easily spread by the wind to generate new infections on neighbouring plants. On infected plants, symptoms begin as small clusters of whitish fungal growth on upper or lower leaf surfaces, which subsequently increase to cover more leaf area, eventually coating the entire leaf with the powdery fungal growth. On markets, there are some resistant lettuce varieties, which represent the best control strategy for this disease. Otherwise, the application of sulphur to leaf surfaces before the onset of disease, when environmental conditions are favourable, can effectively inhibit disease development.

Lettuce can be affected by bacterial diseases and the ones that most commonly occur on this crop are caused by *Xanthomonas campestris* pv. *Vitians* Brown and *Erwinia carotovora* pv. *carotovora* Jones. The bacterial lettuce leaf spot, caused by *X. campestris*, can occur both on leaf and head lettuce and is highly dependent on wet and cool climatic conditions for both infection and disease development. The pathogen is seedborne, therefore, in the case of lettuce seedlings grown as transplants, the pathogen may be brought in field from plants bought from the nursery. Infected plants firstly show small, water-soaked leaf spots, typically bordered by leaf veins and angular in shape, on older leaves. Subsequently, these lesions turn black. This colour is a characteristic

symptom of this bacterial disease. If the disease is serious, several lesions can combine, making the leaf collapse. Usually, lesions don't grow on developing young leaves. This disease can be prevented by using pathogen-free seeds or seedlings to be transplanted. Avoiding sprinkler irrigation can also be a good disease control strategy, as the splashing water from sprinkler irrigation is able to move the bacteria from plant to plant.
Lettuce bacterial soft rots, instead, are caused by *Erwinia carotovora* pv. *carotovora*, a bacteria that occur in the soil and infects through wounds made by insects, or by cultural practices. Infections can also occur through natural openings when water is present on the plant. On infected plants, the first symptoms appear as water-soaked spots, generally on the outer leaves, that form brown slimy areas, before spreading to the lettuce head. These rots cause the outer infected leaves to wilt, eventually spreading to the stem, which results in the collapse of the plant. This disease is favoured by warm and wet climatic conditions; therefore to control the pathogen it is important to avoid the planting in soils that are keen to become waterlogged and to avoid to plant lettuce too close to each other, allowing wind movement. It is also important to pay attention during weeding activity to not to damage the leaves, allowing the entry of bacteria as well as using clean and disinfect equipment during harvest. After harvest, rots can occur in storage when infected leaves are in contact with healthy ones, therefore, it is important to handle plants carefully, avoiding wounding the leaves. Both bacterial diseases cannot be controlled neither with the use of chemical nor biological treatments.

Lettuce also suffers from several viral diseases, including lettuce mosaic virus (LMV) and big vein virus, which are the most common. LMV is a typical potyvirus (genus *Potyvirus*) and is transmitted to lettuce by several species of aphids and by less than 10% by the seed produced by infected plants. It is able to infect all types of lettuce, as well as other members of Asteraceae and several weeds and wild lettuce species. On infected plants, LMV symptoms vary, depending on lettuce cultivar, on plant age when infected, and on environmental conditions. They generally appear as characteristic green and yellowish mottling with mosaic pattern on the leaves of infected plants, which usually, show a downward rolling of the tip. Other symptoms can include leaves yellowing and vein clearing. In some cases, infected plants also fail to form the head, growing irregular leaves. All symptoms altogether result in the infected lettuce plants to be unmarketable. Similarly to other virus-related diseases, there are no means to cure plants from LMV once they get infected. The only control methods are based on prevention, and mainly on the ensuring of the absence of LMV in seed lots before trading and on the correct aphids defence strategy. Big vein virus, instead, is caused by a virus-like agent, that is carried or vectored by the soil-borne and root inhabiting fungus *Olpidium brassicae* Woronin. This fungus produces, under saturated soil conditions, zoospores which are able to transport the pathogen and inoculate it into lettuce root cells. The incidence of this disease is therefore higher in heavy textured and poorly drained soils. The name of the disease is due to its symptoms on leaves, which appear as pronounced clearing of the chlorophyll next to major leaf veins, giving the appearance of enlarged veins, especially when held up to bright light. Moreover, infected plants grow more slowly and may fail to form the head. To manage this disease, there are on the market varieties of lettuce that are tolerant. As for the other virus-related disease, there is no effective chemical soil treatments available.

2.2. *Lygus rugulipennis* (Miridae)

Miridae (Hemiptera: Heteroptera: Cimicomorpha), or plant bugs, is one of the richest in species insect families. Indeed, it counts more than 11,000 species in all over the world (Cassis and Schuh, 2012). From an economic standpoint, in Miridae family, one of the most important genera surely is *Lygus*. In this genus, indeed, some bugs extremely harmful to a varied range of crops are contained (van Emden, 2013). Among the various species which are present in Palaearctic Region, the European tarnished plant bug (*Lygus rugulipennis* Poppius) is the most common and dangerous one found damaging in Europe (Koczor *et al.*, 2012)

2.2.1. Morphology

The European tarnished plant bug, *Lygus rugulipennis*, belongs to a large complex of morphologically similar *Lygus* plant bug species, which includes *Lygus lineolaris*, *L. elisus*, *L. shulli*, and *L. hesperus* among others (Schwartz and Foottit, 1998). In general, *Lygus* bugs can be identified from other genera of the Miridae family by the presence of a shining pronotum, which present deep unobscured, and widely separated punctures, by rounded and convex lateral margin of pronotum, by a deeply punctuated scutellum, and by an oblique head, with linear first and second antennal segment. Study on these different *Lygus* species highlighted a high variability in size, colouration, and characteristic patterns on both pronotum and scutellum among the species. Especially, the high variability of dark patterns can be observed on pronotum and scutellum (Lashkari and Hosseini, 2012).

Lygus rugulipennis can be distinguished from other *Lygus* species by its dimension, usually small (4.5-6.0 mm), by a very dense pubescence in the middle of the corium, with hair length higher than other species (75-80 µm), by very closely spaced punctures in the middle of the corium, and by slender spicula of aedeagus.

Adults range in colour from brownish/reddish grey to greenish grey, depending on the generation, and seldom show two small round spots in the anterior half and a notch in the posterior corners of the pronotum (Fig. 7). Scutellum is mainly characterized by two triangular notches on the front edge, sometimes forming a sort of “W” design (Bei-Bienko and Baghdanov, 1955). The colour of pattern and markings ranges from purple to yellowish brown, and are usually more strongly marked in males, while in females commonly vary from dark red to light reddish-brown. The emielytra often carry small black punctate notches, while the transparent part of the wings is brown with faint light spots. Legs are quite bristly, brown, and characterized by the presence of two dark rings at the thighs.

The antennae are dark coloured with the exception of the second segment, which is lighter coloured in the middle.

At maturity, adults male body reaches 4.7-5.4 mm in length, and female 5.2-5.7 mm, while the body’s width, respect to the length of the second article of the antennae, is equal to 1.3 times in the male and 1.4 times in the female. Moreover, males can be distinguished from females by observing differences on the lower ventral surface of the abdomen, where females show an obvious slit on the rounded abdomen where the ovipositor rests, while the abdomen of males is less round, more pointed near the end, and does not present a slit.

Figure 7 - On the left an adult L. rugulipennis specimen, with its typical color. On the right, a detail of the corium and scutellum.

2.2.2. Host plants

The European tarnished plant bug is a highly polyphagous species, and an important pest of a wide range of herbaceous plants, vegetable crops, commercial flower plants, fruit trees, and nursery stock, throughout Europe (Holopainen, 1986; Kelton, 1975; Wheeler, 2001; Khanjani, 2005). Indeed, this polyphagous bug is reported to attack more than 400 species of plant, belonging to over 50 plant families (Holopainen and Varis, 1991; Taksdal and Sørum, 1971). Its host plant range includes:

- alfa-alfa (Benedek *et al*., 1970; Erdelyi *et al*., 1994)
- wheat (Holopainen, 1989; Varis 1991)
- strawberry (Jay *et al*., 2004; Labanowska, 2007; Bosio and Scarpelli, 1999)
- several cucurbits (Cross, 2004; Jacobson, 2002)
- kenaf (Conti *et al*., 2001)
- peach (Tavella *et al*., 1996)
- lettuce (Accinelli *et al*., 2005; Jacobson, 1999)
- and several others important cultivated crops, such as cereals, potato, sugar beet, brassicas, and carrots (Vappula, 1962; Khanjani, 2007; Varis, 1972; 1995; Dragland 1991a, b) as well as numerous wild plants.

In particular, in the northern Italian region, *L. rugulipennis* is one of the most important pests of lettuce, mainly damaging the transplants performed during summer months (Accinelli *et al*, 2002).

2.2.3. Biology

The European tarnished plant bug overwinters in the adult stage, generally hidden into leaf litter, under bark, or between leaves of herbaceous plants. Overwintering adults usually become active early in the spring, following the rising of the temperature.

As soon as they emerge, they start their feeding activity on the newly developing buds and shoots of spontaneous herbaceous plants. Soon after, as temperature rise until around 20°C, they migrate to other plants, in order to start mating.

Fertilized females start then searching their oviposition site, through the exploration of the possible oviposition site with their mouthparts, performing "probing" behaviour (Romani *et al*., 2005). Once the oviposition site has been identified, eggs are inserted inside plant tissues, creating a wound with the ovipositor. Females generally deposit eggs into stems, petioles, any leaf parts, buds, and flowers of host plants, typically laying few eggs per day. At the optimum temperature for oviposition (20 C) a single female is able to lay over 100 eggs during the entire lifespan.

Eggs are small (around 1 mm), whitish, and slightly curved. At the top, where the egg joins the plant tissue, it presents a flatted opening, which is used by the hatchling nymph to emerge. Depending on the temperature, eggs take between 1 to 2 weeks to hatch.

The European tarnished plant bug, as with all bugs, has no pupal stage, and the life cycle only involves egg, nymph, and adult stages. In particular, this pest develops through five nymphal stages. Newly hatched first instar nymphs are small in dimensions (around 1 mm in length), greenish yellow in colour, and wingless, often mistaken for aphids.

Nymphs also present several characteristic black spots, usually 4 on the dorsal part of the thorax and 1 on the dorsal part of the abdomen, which become more noticeable as nymphs mature through their five instars (Fig. 8). During maturation, nymphs also grow in dimensions, gradually becoming more like adults in appearance within each moult, up to 4.5 mm in length, also developing wing buds in fourth and fifth instar (Slater and Baranowski 1987; Schuh and Slater 1995; Dolling 1991; Kelton 1975).

Each instar lasts about 3-4 days and, typically, the whole life cycle takes 30-40 days, depending mainly on temperature. Indeed, at 34°C *Lygus* may take less than 2 weeks to progress through the five nymphal instars, but at 12°C the time may increase up to 40 days.

Number of generations of *L. rugulipennis* can therefore vary, depending on climatic conditions, from just one generation in Nordic countries (Varis, 1972) to up to several in countries with better climatic conditions (Layton, 1995; George *et al*., 2021).

In northern Italy, *L. rugulipennis* generally presents two annual generations, with the first taking place on spontaneous herbaceous plants, while the second is capable of causing damage to several cultivated plants. This migration to cultivated fields is usually a consequence of cultural practices in the adjacent crops, such as mowing fodder or harvesting winter cereals.

Diapause is induced when nymphs begin being exposed to short days (<12.5 h) and resultant adults enter diapause in order to overwinter.

Figure 8 - Five development instars of nymphs.

2.2.4. Damage

Lygus rugulipennis damage to plants is caused either by its feeding or oviposition activities. When feeding, *L. rugulipennis*, through its piercing sucking mouth parts injects into plants tissues its saliva, which contains several toxic enzymes, such as polygalacturonase, amylase, alkaline protelnase and, to a lesser extent acid protelnase, phosphatase, trehalase, mverlase and phenoloxldase (Laurema *et al*., 1985, Laurema and Varis, 1991; Easterbrook, 2000; Cross *et al*., 2011).

Most crops are damaged by *L. rugulipennis* on stems, vegetative apexes, rib and leaf limb, and flowers, and main feeding damage symptoms include the wilting of the distal part of the shoots (e.g. in apple or peach trees), the flower or seed abortions, the necrosis of the stems or leaves, the branching of the shoot (e.g. in potato or rape), multiple crowns (e.g. in sugar beet or carrot), the malformation of berries (e.g. in strawberry), the arrested shoot growth (e.g. in cabbage or cauliflower) and even the death of the plant in the case of young seedlings. In most of the crops, indeed, the seedlings or the young plants are the most vulnerable stages, therefore main damage is caused by the overwintering adults migrating from weeds.

On lettuce, instead, damage is potentially serious even on older plants, as *L. rugulipennis* adults may be found feeding on the leaves of the maturing crop causing on the internal part of the leaf ribs necrotic spots that subsequently deepen and extend, until they form a blackish furrow that marks large tracts of the rib (Fig. 9). Therefore, unlike most of the other crops, the period in which lettuce suffers the most damage in northern Italy is from the second half of July to the end of September. This is because of the high population density of the pest during those months and because of the scarcity of alternative feeding sources. In summer, indeed, lettuce is one of the few crops in the field able to stay fresh, thanks to frequent irrigation, thus making it highly appetizing for *L. rugulipennis*.

On lettuce, *L. rugulipennis* damage causes directly marketable yield loss, since damaged leaves must be removed before commercialization.

Damage can also occur through oviposition activity, caused by females robust ovipositor which is used to drill into the host plant in order to release the egg inside the vegetative tissues.

Figure 9 - Characteristic damage of L. rugulipennis on lettuce leaf rib.

2.2.5. Control measures

Lygus rugulipennis damage on lettuce is commonly controlled by using preventive permitted insecticidal treatments. Among insecticides, pyrethroids are often prescribed because of their good efficacy against *L. rugulipennis* and other lettuce pests. In Italy, following the Integrated Production Regulation issued by Emilia-Romagna region, the only permitted pyrethroid remained is Etofenprox, and its application on lettuce to control *L. rugulipennis* is linked to the presence in the field as a threshold. These products, however, are generally broad-spectrum and show therefore a negative impacts on both the environment and non-target insects. Their applications can indeed severely damage any biocontrol agent naturally present on the crop, which may be active against either on *L. rugulipennis* or on other pests. Moreover, their use is increasingly being restricted (Hillocks, 2012).

In organic farming regime, since pyrethroids applications are not permitted, growers have just few possible measures against this pest. These measures, which have been studied on several crops mainly in the North America and in the UK on strawberry fields, include:

- release of natural enemies, mainly parasitoid of the genera *Anaphes* (Hymenoptera: Mymaridae) and Peristenus (Hymenoptera: Braconidae) (Haye *et al*., 2005, Norton and Welter, 1996; Pickett *et al*., 2009, Udayagiri, 2000),
- the use of reflective mulch between rows (Rhainds *et al*., 2001),

- the use of vacuuming devices to materially remove the pest (Vincent *et al*, 2000; Rancourt B. *et al*., 2003; Swezey S.L. *et al*., 2007).

Furthermore, some agronomic practices can help reducing *L. rugulipennis* migrations into lettuce fields, thus limiting its damage.

Since generally these migrations occur in summer, driven by agronomic practices carried out in the adjacent crops, such as the mowing of the fodder or the harvesting of winter cereals, it is important to avoid as possible the mowing of drains and meadows next to lettuce fields in the period of July - September.

Another important cultural practice required to limit *L. rugulipennis* infestations is a correct weed control and tillage, which are capable of reducing the availability of host plants.

Lygus rugulipennis can also be partially controlled by several natural enemies. In particular, various species of ladybird beetles, such as *Coccinella septempunctata*, are known to consume third, fourth and fifth instar of the pest, but in low numbers. Contrarywise, several species of damsel bugs (Hemiptera: Nabidae), several *Orius* species, lacewing larvae (Neuroptera: Chrysopidae) and crab spiders (Araneae: Thomisidae) are able to consume greater numbers of *L. rugulipennis* nymphs.

Furthermore, the fungus *Beauveria bassiana* is reported as control agent of wide host range (>700 insect species), which also include *L. rugulipennis*. However, its use is not specifically registered against *Lygus* bugs. Therefore, lettuce growers, especially those under organic farming regime, need alternative approaches for *L. rugulipennis* management and, among these, the introduction of a push pull strategy (which will be deepened in Chapter 2) could prove to be effective.

2.3. Push-pull strategy

Push-pull strategies are pest control systems, which undertake a holistic agroecological approach, exploiting chemical ecology, cultural practices, and agrobiodiversity.

These strategies use a combination of behaviour-modifying stimuli in order to manipulate the distribution and abundance of insect pests and/or natural enemies on a particular protected resource, usually the main crop. Push-pull strategies include insect stimuli that serve to make the main crop unpalatable and unattractive to the pests (push component), while they are simultaneously lured towards a more attractive source, using highly apparent and attractive stimuli (pull component), to other areas such as traps or trap crops where they are concentrated, facilitating their control (Bhattacharyya, 2017).

Push-pull strategy was first conceived as insect pest management strategy in Australia in 1987, were the use of both repellent and attractive stimuli, not deployed alone but in tandem, in order to manipulate the distribution of *Heliocoverpa spp*. in cotton has been investigated (Pyke *et al*., 1987). However, it was only in 1990 that the concept was better formalized and refined, when this type of strategy was named “stimulo-deterrent diversion” (Miller and Cowles, 1990).

Since then, most of the works on push-pull strategies have been directed to modify pest behaviour in order to limit the damage they cause on cultivated crops, even though these strategies could also be targeted to enhance beneficial organisms populations and activities.

Behaviour-modifying stimuli for use in push-pull strategies can be divided into:

Stimuli for Push Components, which include:

- Visual cues, which consist in the manipulation of one or more plant characteristic such as colour, shape, or size in order to reduce pest orientation. Their use is often difficult, so they have rarely been used.
- Synthetic repellents. There are some commercially available that may be used in push-pull strategies to drive away from the main crop several pests.
- Nonhost volatiles, derived from plants that are not in the host plants range of the pest. They can be used to mask host odours or to evoke repellent behaviours.
- Host-derived semiochemicals, instead, exploit the insects ability to identify plant host thanks to key volatiles present in specific ratios. If applied in incorrect ratios pest-host orientation can ceases.
- Anti-aggregation pheromones, used in order to control the spatial distribution of insects through the reduction of intraspecific competition.

- Alarm pheromones, naturally produced by several insect species if attacked, can cause the avoidance or dispersal behaviour in members of same species. This stimulus has been implemented for example in push-pull strategies against many aphid pests (Hardie *et al*., 1999).
- Oviposition deterrents and oviposition deterring pheromones. These are compounds able to prevent or, at least, reduce egg deposition. Therefore, could be of useful implementation in push-pull strategies aimed at controlling pests with harmful larvae or that cause damage through oviposition activity.

Stimuli for Pull Components, which include:

- Visual stimulants, which can include for example traps of different colour, used to enhance the effectiveness of others attractive stimuli.
- Host volatiles, that can be used to bait traps for either monitoring, mass-trapping, or attract and kill strategies.
- Sex and aggregation pheromones. Insects naturally produce sex and/or aggregation pheromones aimed to attract other members of the same species for either mating or improving resource use. These pheromones can be replicated in order to pull pest away from the main crop through, for example, baited traps.
- Gustatory and oviposition stimulants, which can be used, for example, in trap cropping to lure the pest away from the cash crop by sowing a more attractive crop in the field border. These stimulants, therefore, can help to retain in the trap crop area the pest populations.

Both Push and pull stimuli can be deployed through the use of traps, natural products or their synthetic equivalents, vegetative diversification: intercropping and trap cropping, antixenotic or resistant cultivars, and plant induction.

The combination of different stimuli can be different in each push-pull strategy, depending on the controlled pest and on the crop to be protected (Cook *et al*., 2007). Indeed, for the development of an effective and sustainable push-pull strategy a good knowledge of the pest's behaviours and interactions with its plant hosts, conspecifics, and natural enemies is required.

Push-pull strategies in pest management aim mainly to increase the efficacy and sustainability of pest control, while minimizing the negative environmental effects of fertilizer and/or pesticide applications. Even though each single component of the strategy may not be as effective as the application of a broad-spectrum insecticide, through tandem deployment of both push and pull components the efficacy can be enhanced. Furthermore, the components of a push-pull strategy are generally harmless and, therefore, can be combined with biological control. (Khan and Pickett, 2008).

2.3.1. Successful examples of application

Push-pull strategy as a mean of pest management has been successfully implemented against several insect pest.

One of the most famous and successful examples, still highly adopted nowadays, is the push-pull strategy developed in Africa for controlling stemborers on cereal crops, such as the maize stalk borer (*Busseola fusca* Fuller (Lepidoptera: Noctuidae) and the spotted stem borer *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) (Chamberlain *et al*., 2006). Stemborers, indeed, are one of the major limitations in the increase of maize and sorghum production, the principal food, and cash crops for millions of poor people. The push-pull strategy developed for their control involves the combined use of both intercrops and trap crops, aimed at trapping stemborers outside the main crop on highly attractant trap plants (pull component) while pushing them away from the cash crop using repellent intercrops (push component). For this purpose, Napier grass (*Pennisetum purpureum* Schumacher) and Sudan grass (*Sorghum vulgare sudanense* Nees ex. Steudel) were used as trap plants, while molasses grass (*Melinis minutiflora* Palisot de Beauvois) and desmodium (*Desmodium uncinatum* von Jacquin and *Desmodium intortum* Miller) were used as repellent intercrop (Khan *et al*., 2007). Furthermore, molasses grass intercropped with maize also served at increasing stemborer parasitism by a natural enemy, *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae).

Another great example of successful push-pull strategy application in pest management is the one developed, and still now used, to control 2 polyphagous lepidopteran pests, *Helicoverpa armigera* Hübner and *Helicoverpa punctigera* Wallengren, attacking cotton in Australia. In this strategy, neem seed extracts were applied to cotton crop (push component), while an attractive trap crop of either pigeon pea (*Cajanus cajan* L.) or maize was planted alongside the main crop (pull component).

A push-pull strategy has also been successfully developed against *Leptinotarsa decemlineata* Say on potatoes, exploiting its attraction to host plant volatiles. This strategy involved potato rows treated with host plant-based attractant (pull component), sandwiched between rows treated with neem-based antifeedant (push component).

Another successful example of push-pull strategy is the one related to the management of the pea leaf weevil, *Sitona lineatus* L., on beans. In this strategy, a synthetic aggregation pheromone (4-

methyl-3,5-heptanedione) was used as a pull component, while neem-based antifeedant formed the push component of the strategy.

One more example of a successful push-pull strategy is the management of the pollen beetle, *Meligethes aeneus* Fabricius attacking oilseed rape (*Brassica napus* L.). This strategy involves the planting of turnip rape (*Brassica rapa* L.) along the perimeter of oilseed rape field as the pull component, while the push component is represented by the application of nonhost plant volatiles based on lavender (*Lavandula angustifolia* Miller), which can repel *M. aeneus* from the main crop.

Finally, a push-pull strategy is also used in order to manage the onion maggot, *Delia antiqua* Meigen (Diptera: Anthomyiidae). In this strategy, small unmarketable onion bulbs serve as a trap crop to divert oviposition of these flies in the main crop (pull component), while cinnamaldehyde applications are used on the main crop to deter the pest (push component.

Similarly, this technique has been also developed against *Lygus* bugs. In particular, *L. rugulipennis* lends itself well to being controlled using this strategy, as the pest presents both clear feeding preference to be exploited using trap cropping, and commercially available synthetic sexual pheromones, to be exploited as part of a mass trapping technique.

2.3.2. Attractive plants for *L. rugulipennis*

Among the wide plant host range of *L. rugulipennis*, several plant species, such as alfa-alfa (*Medicago sativa* L.), red clover (T*rifolium pretense* L.), mugwort (*Artemisia vulgaris* L.) and sunflower (*Helianthus annuus* L.), could be implemented as trap crops (pull component in a push-pull strategy) for *Lygus spp*. management in lettuce, due to their higher attractive power (Accinelli *et al*. 2005; Ondiaka *et al*., 2016; Rämert *et al*., 2001).

In particular, alfa-alfa represents one of the most studied crops for the use as a trap crop against this pest, especially to protect strawberries. Most of the studies highlighted a major abundance of both *Lygus* adults and nymphs on alfa-alfa trap crop compared with nearby strawberries (Swezey *et al*., 2007; Swezey *et al*., 2013). *Lygus* bugs' attraction, indeed, is driven by volatile plant odours present alfa-alfa, which are highly attractive to females, affecting their movement and therefore also that of males (Blackmer *et al*., 2004; Godfrey and Leigh, 1994).

However alfa-alfa trap crops alone, although highly attractive for *Lygus* bugs, demonstrated that they cannot prevent pest migration to main crop during periods of high population density (Pansa and Tavella, 2009). To overcome this issue, some studies have also evaluated the effectiveness of alfa-alfa trap crop borders, subsequently treated with insecticides, showing good results (Accinelli *et al*., 2005), while a more environmentally friendly alternative is represented by localized release of *L. rugulipennis* natural enemies on alfa-alfa borders, where they find ideal living conditions, thus intensively reproducing and successfully reducing the impact of the pest. Anyway, both these strategies failed to effectively control pest damage on lettuce in periods in which the populations of *L. rugulipennis* are particularly high (July-September).

A further alternative could be represented by a semiochemical-assisted trap cropping strategy based on the use of both alfa-alfa trap-crop borders and traps baited with sex pheromones for mass trapping, which will be deepened in Chapter 2.3.3 and 2.3.4.

2.3.3. Type of traps

The traps used for the monitoring of *L. rugulipennis* are called Green cross vane Unitrap. Each trap consists in:

- A bucket to collect the insects of approximately 16 cm of diameter and 12.5 cm of height.
- A funnelled entrance, with a 3 cm wide opening at the bottom and around 10 cm wide opening at the top.
- Cross-vanes inserted above the funnel top opening, characterized by a 12 cm width at the bottom, 14 cm at the top and a 11 cm height.
- A circular lid inserted above the cross vanes, of approximately 16.5 cm diameter width, to prevent rain from falling into the trap.
- A container equipped with bars, which is placed in a hole under the middle of the lid and extends into the middle of the cross-vanes (highlighted in red in Fig. 10).
- The attractive pheromone is placed inside the container.
- As killing agent, inside the bucket of the trap either water and detergent or a piece of net impregnated with insecticide can be placed.

The traps were fastened to a white signpost and lean on the ground in order to prevent them from being moved by the wind.

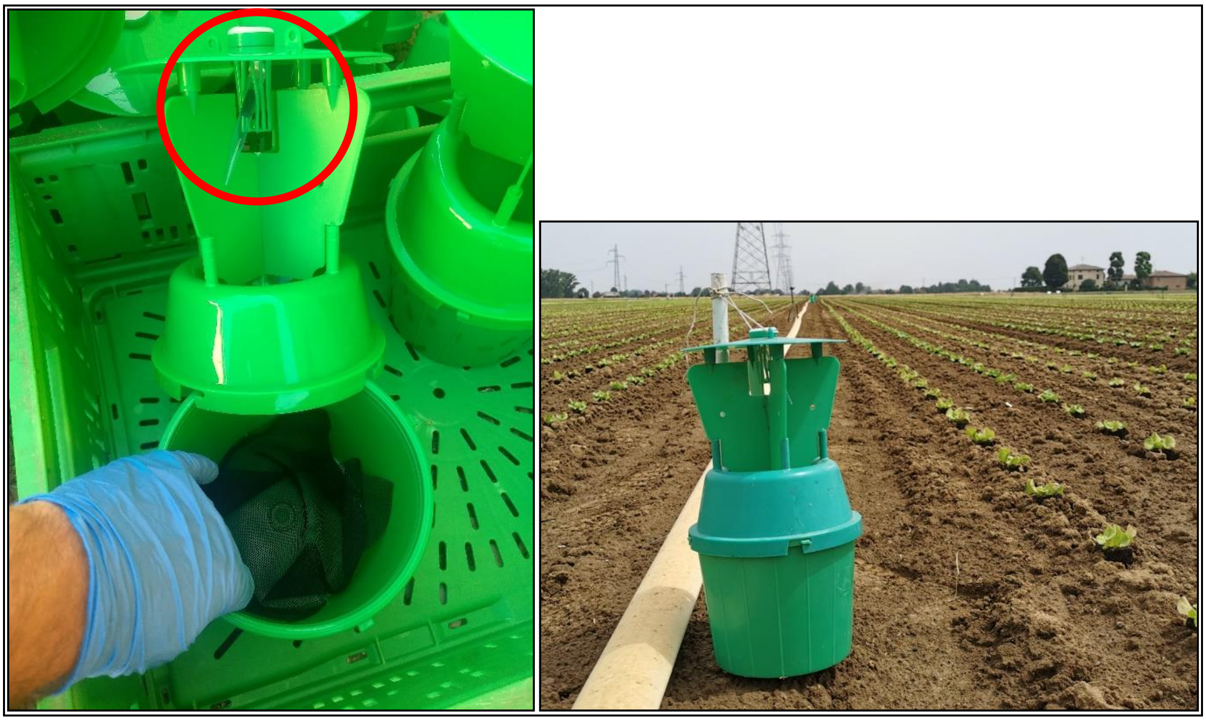

Figure 10 - Detail of the pheromone container (left photo) and of the system for fixing the trap to the ground (right photo).

2.3.4. Semiochemicals

Nowadays, push–pull strategies highly rely on reproducing insect and/or plant natural interactions to deter pest incursion into crops and attract them away from cash crops through synthetic insect- and plant-produced semiochemicals (Fountain *et al*., 2021).

In particular, for *L. rugulipennis*, a specific synthetic female-produced sex pheromone has already been developed and tested. This pheromone is composed of a specific blend of substances which are naturally released by *L. rugulipennis* females and are known to stimulate males' antennae. The composition of the blend is Hexyl Butyrate, (E)-2-hexenyl butyrate and (E)-4-oxo-2-hexenal in the rate 100:3:20 (Fountain *et al*., 2014; Innocenzi *et al*., 1998; Innocenzi *et al*., 2004).

This pheromone blend demonstrated, if combined with Green cross vane Unitrap, to effectively attract adult *L. rugulipennis* males, with all three compounds required for maximum attractiveness., therefore highlighting a potential use as a monitoring tool (Baroffio *et al.*, 2018; Fountain *et al*., 2017; Innocenzi *et al*., 2005).

The pheromone blend is generally impregnated onto a cigarette filter, inserted in a polypropylene pipette tip.

Furthermore, *L. rugulipennis*' females have also highlighted to be attracted to a plant floral volatile component, phenylacetaldehyde (PAA), which could be potentially used to improve the attractiveness of the pheromone blend baited traps (Fountain *et al*., 2010; Koczor *et al*., 2012). The pheromones used during the tests were supplied by Massimo Dal Pane of the Isagro company.

3. *AIM OF THE WORKS*

The general objective of this work is to test and evaluate the effectiveness of agroecological strategies in the management of insect pests damaging important crops present in northern Italy.

In particular, regarding flea beetles, the specific aim of the study was to develop an agroecological approach to manage their infestations on sugar beets by using attractive trap-crops, thus avoiding a massive use of broad-spectrum insecticides. This strategy, if effective, could lead to an important reduction of chemical inputs, while enhancing the biodiversity and the resilience of the agroecosystems. Furthermore, this agroecological strategy could represent the suitable practice for managing flea beetles infestations in organic farm, where preventive methods are particularly recommended.

The other specific aim was to evaluate the efficacy of a semiochemical-assisted trap cropping strategy based on the use of pheromone baited traps adjacent to attractive alfa-alfa trap-crop borders for the management of *Lygus rugulipennis* infesting lettuce, which minimize insecticide use for the control of an important economic pest. This strategy could be referred to as push-pull strategy, however the term would be improperly used, since the push component has been replaced by the occurrence of two pull stimuli, represented by the alfa-alfa borders and by the presence of traps triggered with pheromones installed inside them. For this reason, the strategy will be referred to as semiochemical-assisted trap cropping strategy. Beside the projects based on bottom-up habitat management techniques at farm scale, a field trial was carried out to test the attractiveness of pheromone baited traps for *L rugulipennis*.

4. *MATERIALS AND METHODS (FLEA BEETLES ON SUGAR BEET)*

4.1. Site description (2020, 2021)

The experimental trials were carried out in both 2020 and 2021 growing seasons in four farms, all located in the north-eastern part of the Emilia-Romagna region, under the province of Ferrara, Northern Italy (Fig. 11).
The farms were called:

- Farm Rossi Albino, located in the eastern part of Codigoro municipality.
- Farm Badile Francesco, located in the western part of Codigoro municipality.
- Farm Delta s.s., located in Tresignana municipality.
- Farm Bergonzini Sandro, located in the eastern part of Copparo municipality (Fig. 12).

The farms were all characterized by having large sugar beets fields, with extensions of minimum 10 hectares.
In 2020th growing season, the sowing of sugar beets was carried out between the last week of February (in the farms Bergonzini and Badile) and the first half of March (in the farms Rossi and Delta).
Sugar beets were sown with 0.45 x 0.15 m spacing between plants in the farms Rossi, Badile and Delta, while in the farm Bergonzini the spacing between plants was 0.45 x 0.16 m.

In 2021st growing season, the sowing of sugar beets was carried out between the end of February (in the farms Bergonzini and Badile), the beginning of March (in the farm Delta) and the last week of March (in the farms Rossi).
Sugar beets were sown with the same densities of the previous growing season in all of the farms.
Farms' coordinates are reported in Table 1, and both 2020 and 2021 trial's fields are shown in Figures 13-20.

Table 1 - Farm locations, sugar beet cultivars, sowing dates and densities.

Farm name	Site	Year	Trial field coordinates	Sowing dates	Sugar beet cultivars	Sowing density
Rossi Albino	Codigoro, 44021 (FE)	2020	44°49'24.76"N 12°12'12.95"E	16 March	Smart Briga KWS (KWS)	0.45 x 0.15 m
		2021	44◦ 49′ 24.1"N 12◦ 12′ 16.9"E	25 March		
Badile Francesco	Codigoro, 44021 (FE)	2020	44°48'14.57"N 12°1'26.91"E	28 February	Bali (SESVanderHave)	0.45 x 0.15 m
		2021	44◦ 48′ 33.4"N 12◦ 04′ 25.7"E	25 February		
Società Agricola Delta s.s.	Tresignana, 44039 (FE)	2020	44°49'52.33"N 11°55'9.65"E	02 March	Bali (SESVanderHave)	0.45 x 0.15 m
		2021	44◦ 49′ 39.0"N 11◦ 54′ 11.7"E	03 March		
Bergonzini Sandro	Copparo, 44030 (FE)	2020	44°51'56.45"N 11°53'22.66"E	25 February	BTS 555 (BETASEED)	0.45 x 0.16 m
		2021	44◦ 55′ 55.6"N 11◦ 52′ 49.2"E	27 February		

Figure 11 - Location of the farms.

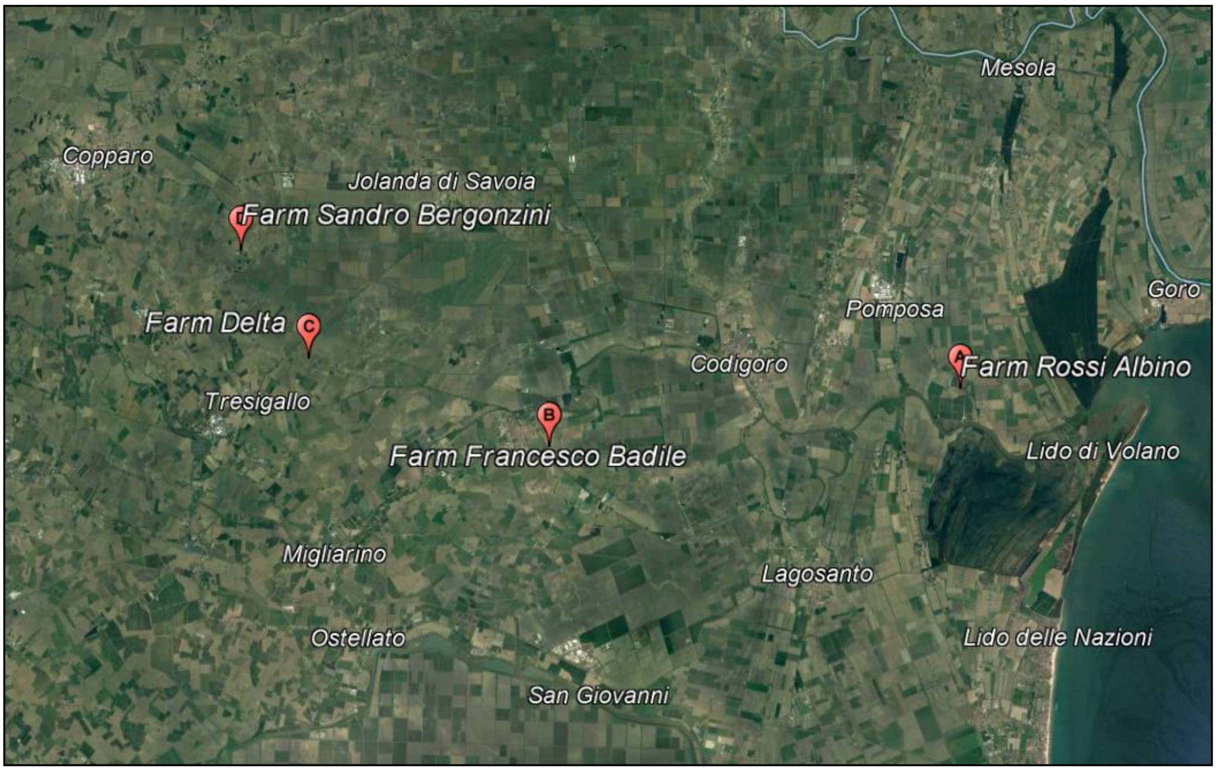

Figure 12 - Municipalities of the farms.

Figure 13 - Field trial in the farm Rossi, growing season 2020.

Figure 14 - Field trial in the farm Rossi, growing season 2021.

Figure 15 - Field trial in the farm Badile, growing season 2020.

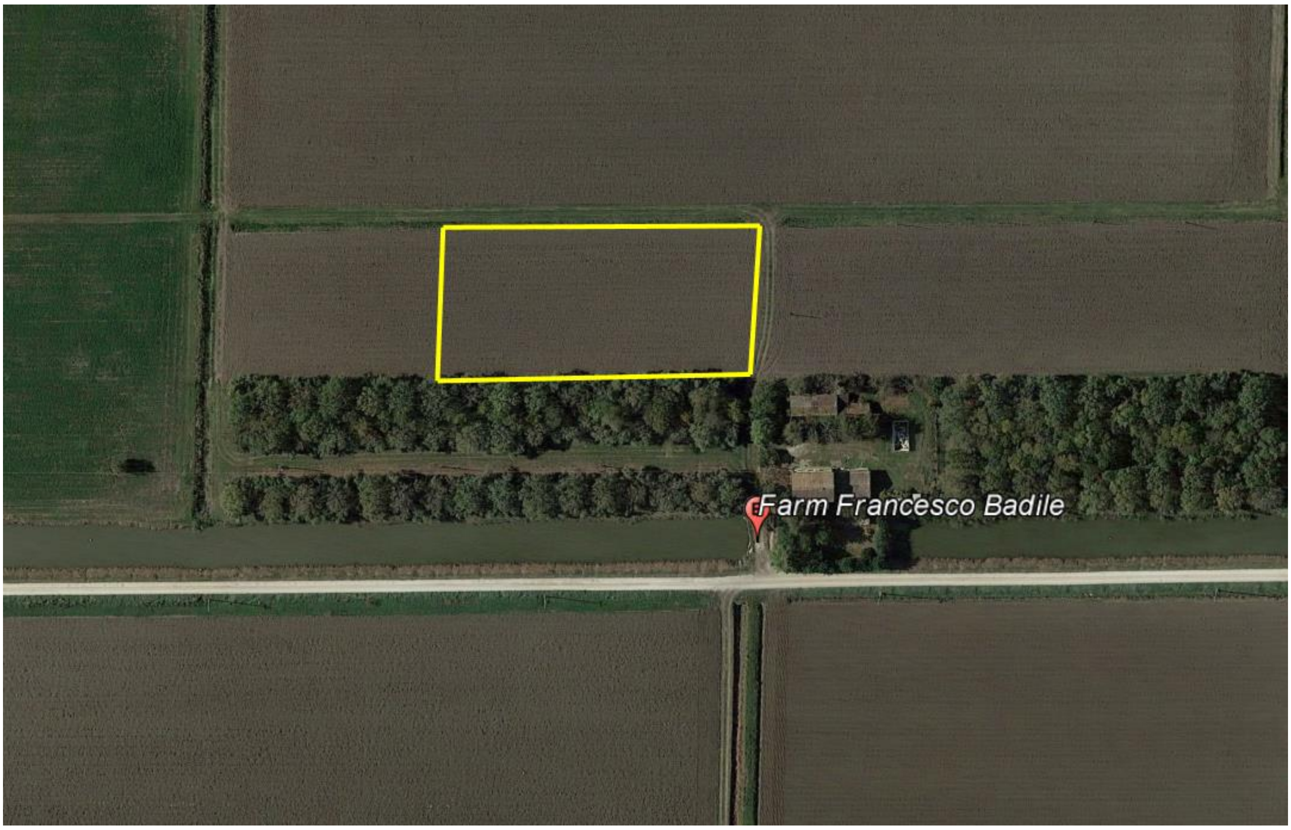

Figure 16 - Field trial in the farm Badile, growing season 2021.

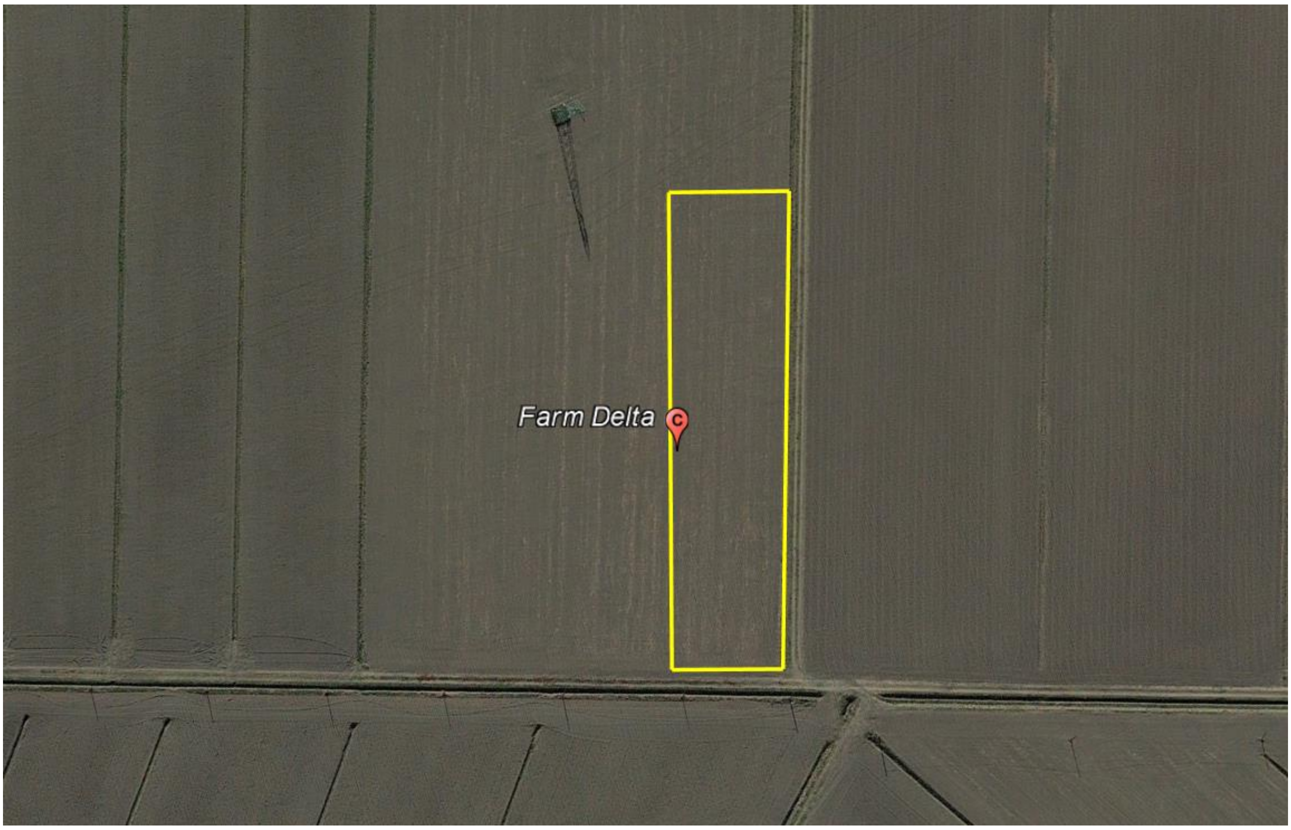

Figure 17 - Field trial in the farm Delta, growing season 2020.

Figure 18 - Field trial in the farm Delta, growing season 2021.

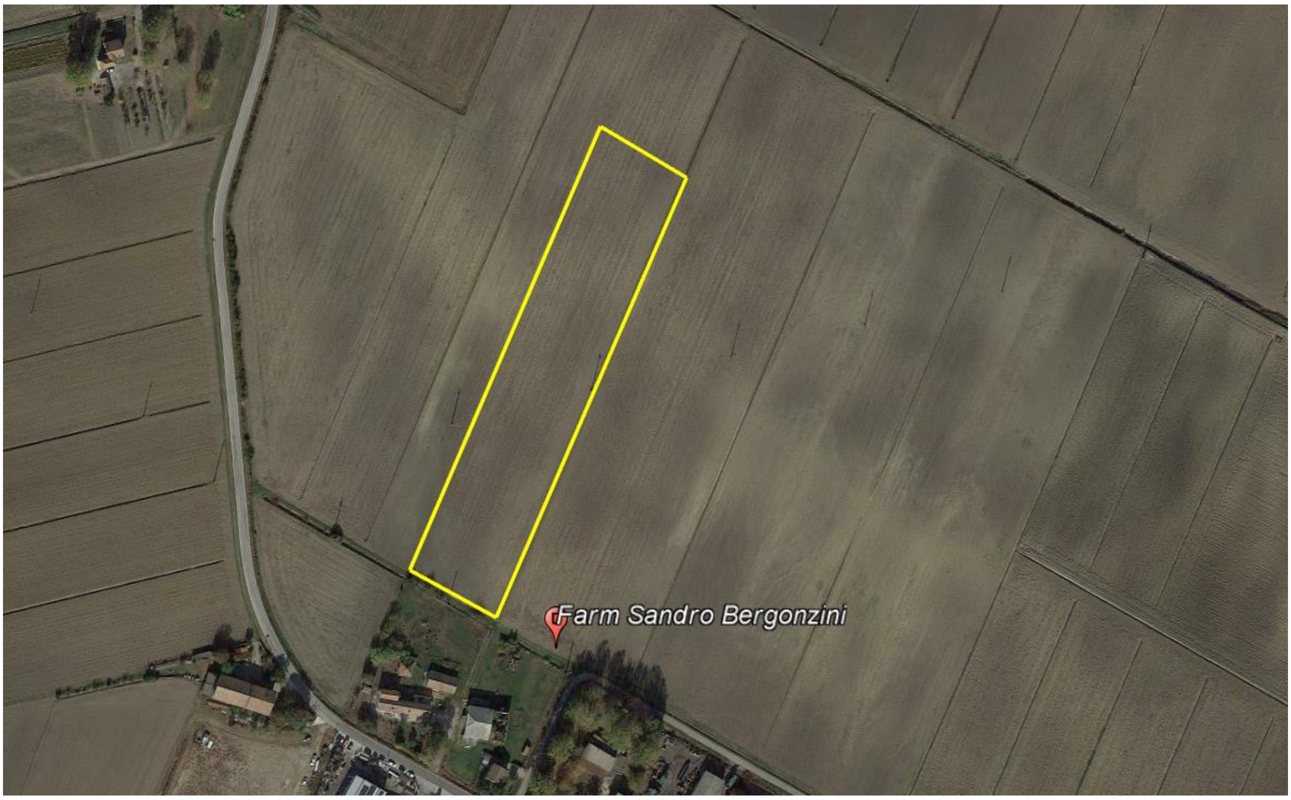

Figure 19 - Field trial in the farm Bergonzini, growing season 2020.

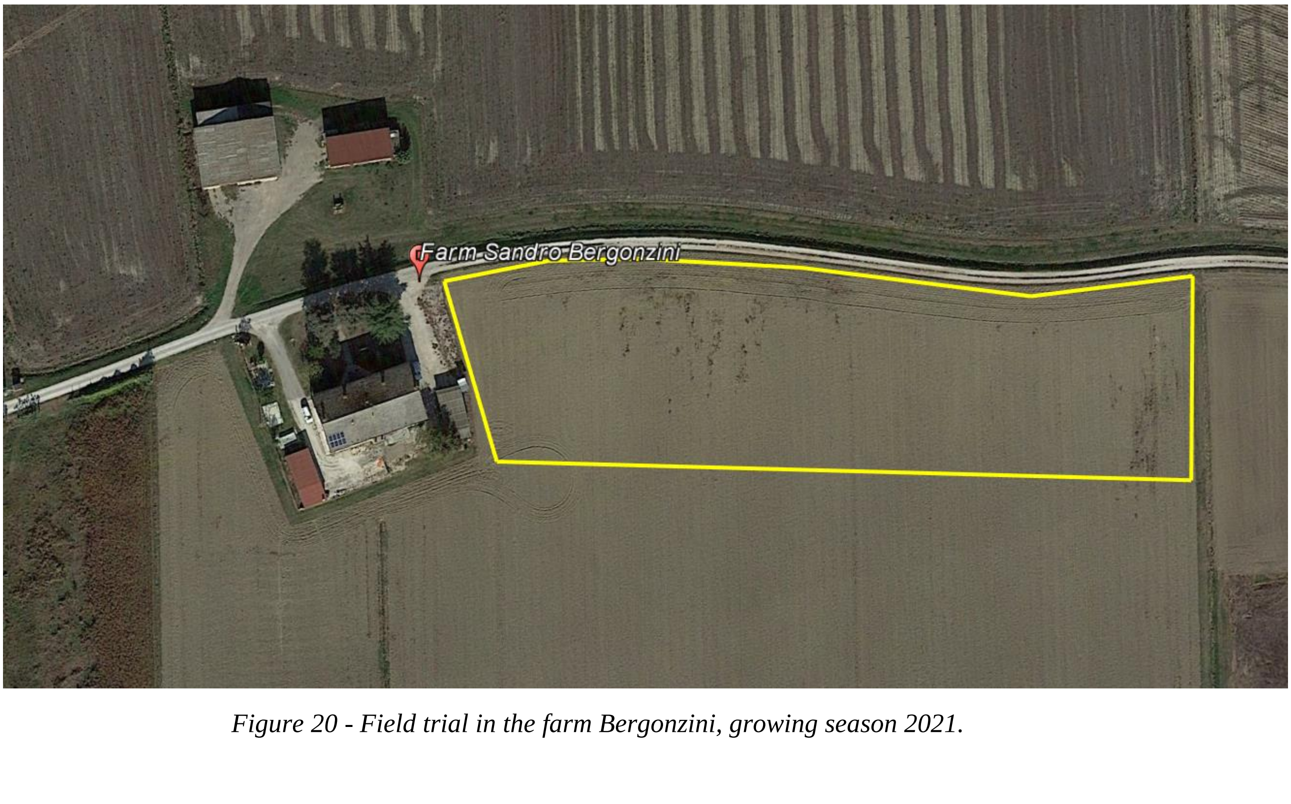

Figure 20 - Field trial in the farm Bergonzini, growing season 2021.

4.2. Experimental design

In each farm, two treatments were compared:

- Trap crop. It consisted in a plot of sugar beet, characterized by the sowing, on one or more edges of a trap crop border.
- Control. It consisted in another plot of sugar beet, characterized by being surrounded only by bare soil.

In each of the farms, the size of both Trap-crop and Control plots was 150 m^2 (10 m length x 15 m width).
The distance between the two plots was at least 60 m, in order to exclude, in the Control plot, the attractive effect exerted by the trap-crop border.

Sugar beet sowing dates are reported in Table 1.

Gold Crop mix (SIS, Societa` Italiana Sementi, Bologna, Italy) was selected as trap crops and sown in each farm a few days before the sowing of sugar beets along one of the field borders, with a mean length of 20 m (range 15–30 m) and a width of approximately 2 m. This mixture, which is composed of 60% Sinapis alba (variety Iris) and 40% Brassica juncea (variety Scala), was selected as trap-crop, due to these plants ‘attractiveness to flea beetles, and to their low-demanding agronomic management.
In both growing seasons, the trial followed a randomized block scheme, where each farm represents a block and hosts a replica.

4.3. Sampling methods

To assess the efficacy of the trap-cropping technique in controlling flea beetle infestations on sugar beets, insect samplings and damage assessments were planned from the beginning of April till the end of May in both growing seasons.

The samplings were carried out on a weekly basis and aimed at assessing the infestations of flea beetles on sugar beets both on the trap-crop treatment plot and on the control plot, for each farm.

In each visual survey, indeed, flea beetle's infestation on sugar beet rows near the trap-crop border (in the trap-crop plot) was compared to the one recorded on sugar beet rows of the control plot, surrounded by bare soil.

In particular, in each farm, the experimental units consisted of a 10 m section of 2 adjacent sugar beet rows parallel to the field border.
The couple of rows were selected based on the increasing distance from either the trap-crop border (in trap-crop treatment) or the bare soil edge of the field (in control treatment). In particular were selected (Fig. 21):

- 2 adjacent rows at a distance of 2 meters from the border/edge,
- 2 adjacent rows at a distance of 6 meters from the border/edge,
- 2 adjacent rows at a distance of 12 meters from the border/edge,
- 2 adjacent rows at a distance of 15 meters from the border/edge.

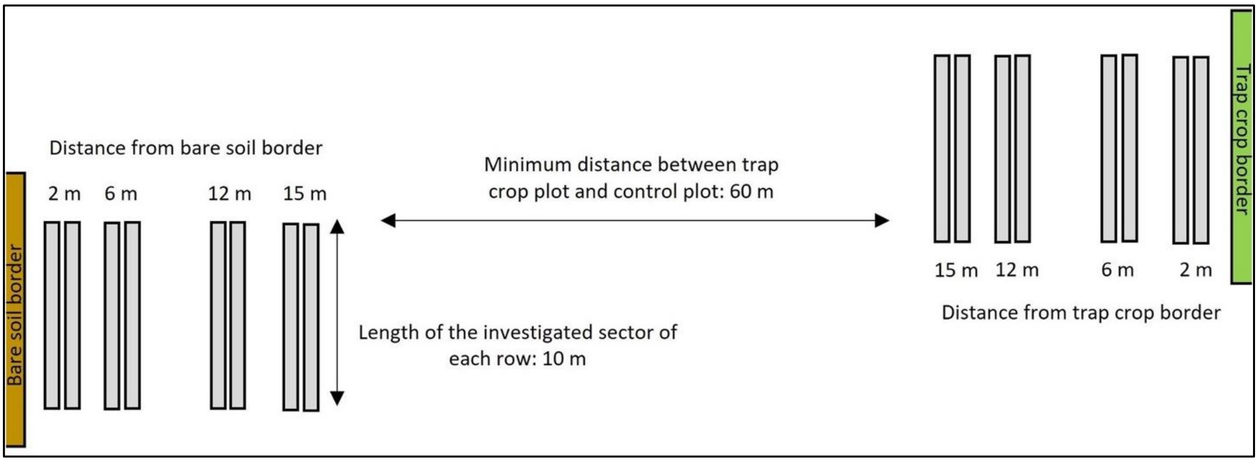

Figure 21 - Experimental design of the trials carried out in both 2020 and 2021 growing seasons.

All the plants of the 10 m section were sampled at each survey in order to verify whether flea beetles, with their feeding damage, did not caused the death in any of the seedlings. Because of different sowing and emergence rates, the total number of plants varied from a minimum of 36 to a maximum of 136 pooling the two rows.
Flea beetle damage on sugar beets is characterized by the presence of small roundish erosions of approximately 1 mm in diameter on the leaf blade, which can merge to form larger holes on the

leaf in the case of heavy infestations. Therefore, feeding damage was evaluated by counting the number of holes caused by the feeding activity of the flea beetles on each leaf of the sampled plants. Each plant was classified into six damage classes depending on the mean number of holes per leaf.

The adopted damage classes were (Fig. 22):

- Class 0 seedlings: Absence of feeding holes, completely healthy plant
- Class 1 seedlings: Seedling with 1-2 holes, limited to 1 or 2 leaves (< 1 feeding hole/leaf)
- Class 2 seedlings: Seedling with the presence of 1 feeding hole on all the leaves
- Class 3 seedlings: Seedling with the presence of 2 feeding holes on all the leaves
- Class 4 seedlings: Seedling with the presence of 3 feeding holes on all the leaves
- Class 5 seedlings: Seedling with the presence of 4 or more feeding holes on all the leaves.

Figure 22 - Details of several damage classes adopted: Class 0 on the left, Class 4 in the middle, and class 5 on the right.

Phenological growth stages were recorded using the BBCH scale to help standardize sampling of each field to specific growth stages, because of different sowing dates at each farm. Three visual samplings were carried out at the following BBCH phenological stages (Meier *et al*., 1993).

- BBCH 12 - 2 leaves (1st pair of leaves) unfolded
- BBCH 14 - 4 leaves (2nd pair of leaves) unfolded
- BBCH 16 - 6 leaves unfolded.

4.4. Statistical analysis

The 10 m sections of two adjacent rows of sugar beets at each distance from the field border were considered as replicates. An index of damage caused by flea beetles was calculated using the following formula (Townsend and Heuberger, 1943):

$$damage\ index\ (\%) = \frac{\sum v\, \mathrm{N}_v * v}{(n-1) * N_t} * 100$$

where:
n is the number of damage classes
Nv is the number of plants in each class of damage
v is the value of the different classes of damage (from 0 to 5)
Nt represents the total number of plants sampled.

Data were firstly analysed separately by year but were then pooled as trends were similar in both years.
A generalized linear mixed model (GLMM) was used to test the effects of treatments (trap crop vs control), distances from border (2, 6, 12 and 15 m) and BBCH stages of sugar beet plants on flea beetle damage index. Gamma probability distribution with log link function was selected because of the positive skewed distribution of residues. The gamma distribution model was also corroborated by the smaller value of Akaike information criterion in comparison with models based on different distributions.
In the GLMM, treatments and distances were considered as fixed factors, while BBCH stages of sugar beet plants were included as repeated measures. Rows were nested within farms and farms were nested within years. A first-order autoregressive covariance structure was selected to model the correlation between repeated measures taken over time in the same experimental unit.
The interactions between factors were included in the model. When a significant effect of a factor with more than two levels was detected, multiple comparisons with Bonferroni sequential adjustment were run ($P < 0.05$).

Analyses were carried out with IBM SPSS Statistics (ver. 26).

4.5. Meteorological data

Meteorological data relating to the period in which the tests were carried out in the eastern Ferrara province are reported in Figg. 23 and 24:

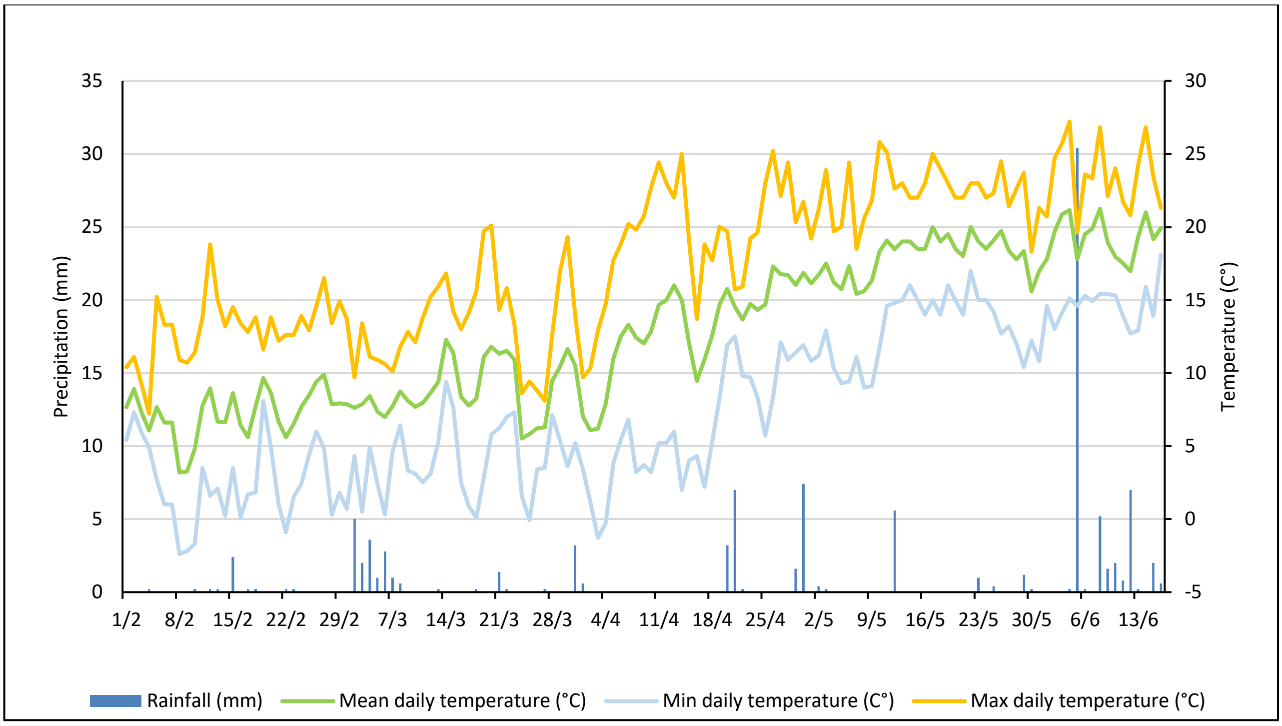

Figure 23 - Meteorological data, growing season 2020.

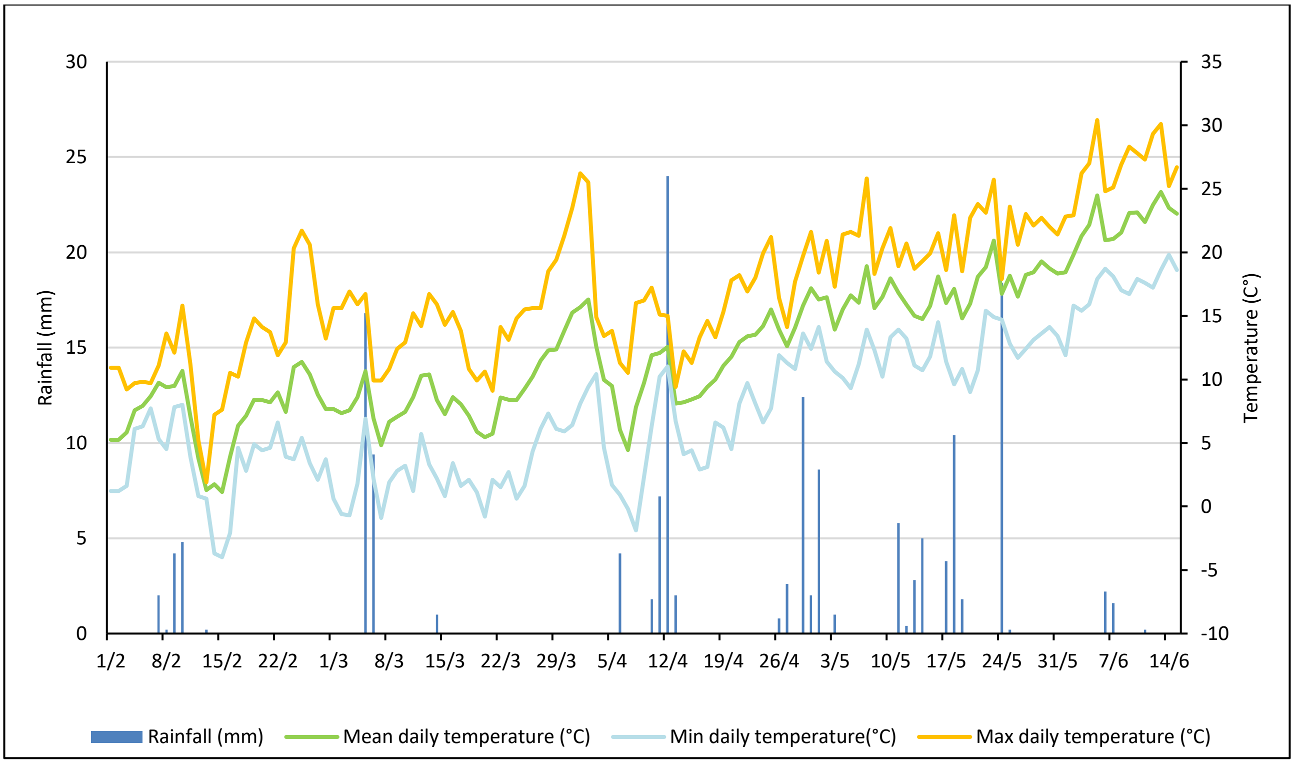

Figure 24 - Meteorological data, growing season 2021.

5. *MATERIALS AND METHODS (Lygus rugulipennis on lettuce)*

5.1. Site description

A) Trials on pheromones effectiveness and traps placement

This trial was carried out in order to evaluate which was the traps configuration capable of expressing the maximum attractive power towards *L. rugulipennis* on lettuce.

This trial was carried out in 2021 growing season, in a farm named Ratta Piero, located in San Lazzaro di Savena, under the province of Bologna, Emilia-Romagna region, Northern Italy.

Trial was carried out on three successive transplant of open field lettuce (*Lactuca sativa* var. *secalina*) (Fig. 25). Lettuce was transplanted with a 0.35 x 0.25 m spacing between plants and all lettuce fields were equipped with sprinkler irrigation system.

Farm coordinates were 44°27'48.09''N and 11°28'14.23''E.

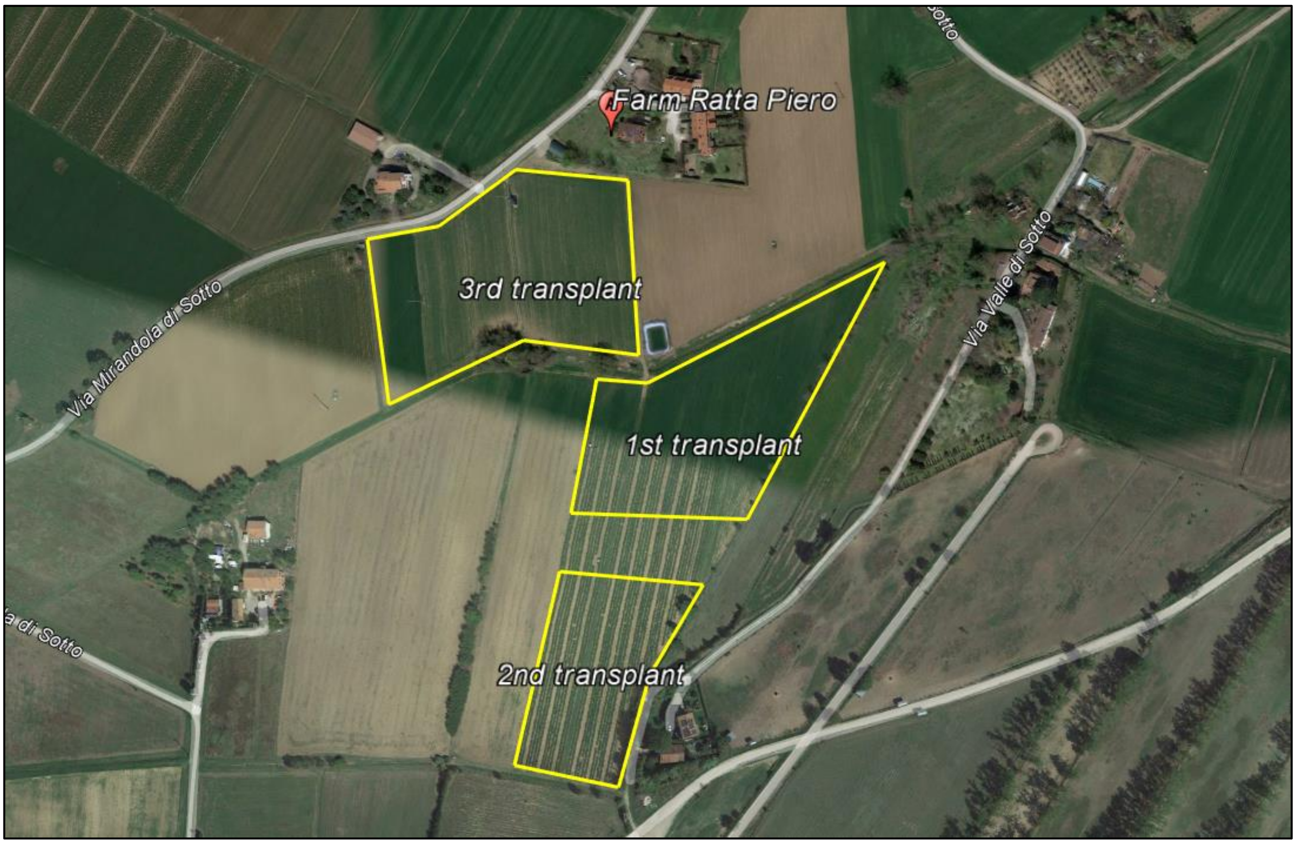

Figure 25 - Field trial in the farm Ratta, growing season 2021.

B) Semiochemical-assisted trap cropping

This trial, aimed at the efficacy evaluation of the abovementioned semiochemical-assisted trap cropping strategy in controlling *L. rugulipennis,* was carried out in both 2020 and 2021 growing seasons.

Trials were carried out in both 2020 and 2021 growing seasons, in the farm named Tonelli Gianni, located in San Lazzaro di Savena, under the province of Bologna, Emilia-Romagna region, Northern Italy.

Similarly to the trial on pheromones effectiveness and on traps placement, also this study was carried out on three successive transplant of open field lettuce (*Lactuca sativa* var. *secalina*) in both growing seasons (Fig. 26 and 27). Lettuce was transplanted with a 0.35 x 0.25 m spacing between plants and all lettuce fields were equipped with sprinkler irrigation system.

Farm coordinates were 44°28'11.4"N and 11°27'43.8"E.

Figure 26 - Field trial in the farm Tonelli, growing season 2020.

Figure 27 - Field trial in the farm Tonelli, growing season 2021.

5.2. Experimental design

A) Trials on pheromones effectiveness and traps placement

In 2021 growing season, four treatments were compared:

- Pheromone baited traps (high), consisting in traps baited with pheromones placed about 70 cm high.
- Pheromone baited traps (low): consisting in traps baited with pheromones placed at ground level (Fig. 29).
- Unbaited traps (high): consisting in traps without pheromone placed about 70 cm high.
- Unbaited traps (low): consisting in traps without pheromone placed at ground level.

For each treatment, 3 Green cross vane Unitrap were installed, for a total of 12 traps. The traps were installed at least 30 meters away from each other, as reported in the scheme in Fig. 28.

The trial followed a randomized block scheme, with three blocks corresponding to the three successive transplants of lettuce. Lettuce transplants were performed in the months of June, July, and August.
After each new lettuce transplant, traps were moved from previous transplant and installed in the new trial field, applying a random rotation of their position, in order to account for the randomized blocks scheme.
The area occupied by each lettuce transplant was approximately 6000 m^2 (100 x 60 m).

In both baited high/low treatments, the pheromone used in this trial was the abovementioned pheromone blend composed by Hexyl Butyrate, (E)-2-hexenyl butyrate and (E)-4-oxo-2-hexenal. Pheromones were replaced with new ones approximately every 30 days.
Furthermore, in order to ensure to kill the captured pests, inside the bucket of all the traps, even those without the trigger, a piece of net impregnated with a substance with an insecticidal action, permitted in organic farming, was introduced.

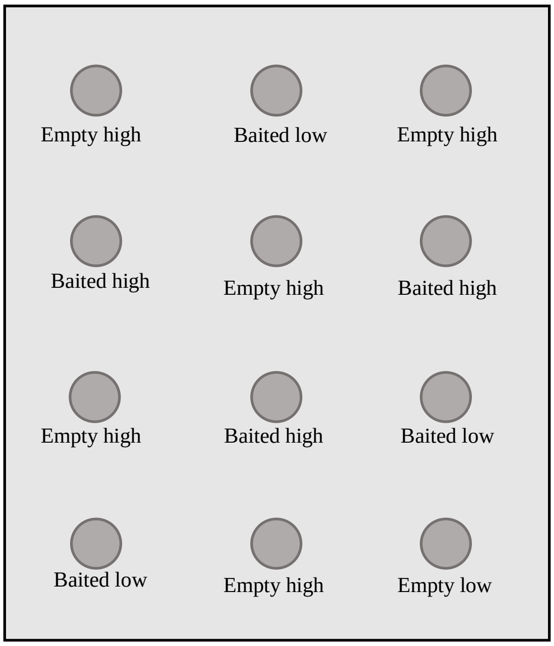

Figure 28 - Experimental statistical design for the first transplant, 2021 growing season (four treatments).

Figure 29 - Pheromone trap in low position

B) Semiochemical-assisted trap cropping

In this study, in both growing seasons, two treatments were compared:

- Semiochemical-assisted trap cropping strategy. It consisted in a plot of lettuce, surrounded on both sides in length by alfa-alfa trap-crop borders. Inside each trap-crop border, 3 traps baited with attractive pheromones were installed.
- Control. It consisted in another plot of lettuce, surrounded by bare soil. In this treatment no traps baited with pheromones were installed.

In semiochemical-assisted trap cropping strategy treatment, the trap-crop plant chosen for the sowing of the borders was alfa-alfa, due to its high attractiveness to *L. rugulipennis* and for its easy management.

In 2020 growing season, alfa-alfa was sown on both edges of the semiochemical-assisted trap cropping strategy treatment plot as borders with a length of 50 m and a width of 2 m. Therefore, the area occupied by alfa-alfa borders was 200 m^2 (50 m in length x 2 m in width x 2 borders). In 2021 growing season, instead the area occupied by alfa-alfa borders increased at 240 m^2, as the length of each border was 10 m higher than in 2020 (60 m in length x 2 m in width x 2 borders)

In this treatment, 3 Green cross vane Unitrap were installed into each of the two alfa-alfa borders, for a total of 6 traps, both in 2020 and 2021 growing seasons (Fig. 30).

In both treatment plots, trial's replicates corresponded to three successive lettuce transplants, which were performed in both growing seasons in the months of June, July, and August (Fig. 31).

The area occupied by each treatment was 750 m^2 (15 x 50 m) in 2020 growing season and 900 m^2 (15 x 60 m). A distance of about 40 m was maintained between the two treatments, in order to avoid any possible interferences caused by the attractive power of either the pheromone baited traps or the trap-crop borders.

Even in this trial the pheromone blend used to trigger the traps was composed by Hexyl Butyrate, (E)-2-hexenyl butyrate and (E)-4-oxo-2-hexenal.

Pheromones were replaced with new ones approximately every 30 days.

The killing method chosen to ensure to kill the captured pests was, also in this trial, the piece of net impregnated with a substance with an insecticidal action, permitted in organic farming, introduced into traps' buckets.

Figure 30 - Alfa-alfa trap-crop border, with pheromone baited trap.

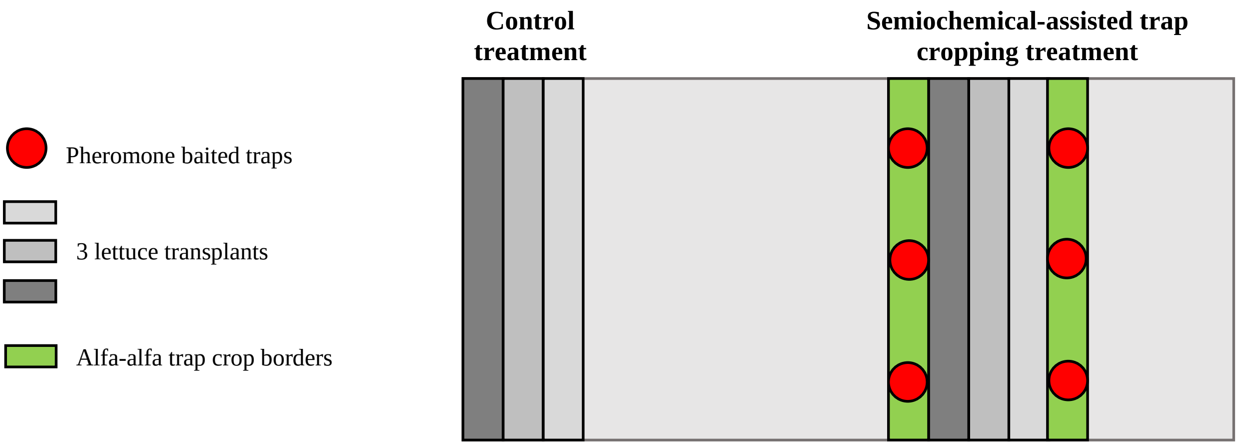

Figure 31 - Semiochemical-assisted trap cropping- Experimental statistical design in both 2020 and 2021 growing seasons.

5.3. Sampling methods

A) Trials on pheromones effectiveness and traps placement

In 2021 growing season, traps were installed on the 24th of June in order to coincide with the first lettuce transplant. At the time of installation, each of the traps was identified by a progressive number, which was also written on the body of the trap, in order to facilitate subsequent emptying operations.

Afterwards, the emptying of the traps was carried out starting from the 8th of July and continued fortnightly until the end of September (28 September). Traps emptying dates are reported in Table 2. The first movement of the traps, in correspondence of the second transplant, was carried out on the 19th of August, while the last movement of the traps, in correspondence of the third transplant, was carried out on the 14th of September.

At each emptying, the piece of net impregnated with insecticide was removed and the insects killed were collected into a plastic bag for insect collecting. Each bag was identified with the number corresponding to that reported on the body of the trap from which the insects were taken.

Subsequently, all bags with collected insects were taken in laboratory.

Subsequently, all the bags containing the collected insects were taken to the laboratory for counting operations. For each bag, the total specimens of *L. rugulipennis* (separated by male and female) were counted. In addition, all specimens of beneficial insects (especially adult Coccinellidae) eventually captured were also counted.

Both the trigger pheromones and the insecticidal net were replaced approximately every 30 days.

Table 2 - 2021 growing seasons activity log.

2021 Dates	2021 Activities
24/06/2021	Traps installation
08/07/2021	1st Emptying of traps
22/07/2021	2nd Emptying of traps
05/08/2021	3rd Emptying of traps Replacement of both nets and pheromones Moving of the traps to new transplant
19/08/2021	4th Emptying of traps
31/08/2021	5th Emptying of traps Replacement of both nets and pheromones Moving of the traps to new transplant
14/09/2021	6th Emptying of traps
28/09/2021	7th Emptying and remotion of traps

B) Semiochemical-assisted trap cropping

2020

In 2020 growing season, into each alfa-alfa border, in the semiochemical-assisted trap cropping strategy, 3 Green cross vane Unitrap, baited with the pheromone, were installed on the 24th of June.
Traps were emptied following the same methodologies reported in the before mentioned trial, starting from the 2nd of July, and continuing on a weekly basis until traps remotion, occurred on the 24th of September (Table 3).

Additionally, in semiochemical-assisted trap cropping strategy trial, samplings on damage were carried out. Indeed, in order to evaluate the effectiveness of this strategy in limiting *L. rugulipennis* damage on lettuce, visual surveys were carried out on the percentage of damaged leaves, both in the control and semiochemical-assisted trap cropping strategy plots.
In particular, for each of the three lettuce transplants, all present leaves on 25 lettuce heads / treatment were counted and divided into healthy or damaged, also diversifying the % of damage (= damaged leaves / total leaves * 100) on the head as it is at harvest (whole head) and on the head cleaned and ready for marketing (commercial head). As damaged, only the leaves damaged by *L. rugulipennis* were considered, while those damaged by other organisms were evaluated as healthy. Furthermore,

2021

In 2021 growing season, in the semiochemical-assisted trap cropping strategy, the installation of the 6 Green cross vane Unitrap was carried out on the 1st of July. The emptying of the traps started the 8th of July and continued weekly until the 29th of September (Table 3).
In 2021, the same methodologies followed in previous year were adopted, for both the activities of insect collecting and damage samplings.

Table 3 – Semiochemical-assisted trap cropping- 2020 and 2021 growing seasons activity log.

2020 Dates	2020 Activities	2021 Dates	2021 Activities
24/06/2020	Traps installation on alfa-alfa borders	01/07/2021	Traps installation on alfa-alfa borders
02/07/2020	1st Emptying of traps	08/07/2021	1st Emptying of traps
09/07/2020	2nd Emptying of traps	15/07/2021	2nd Emptying of traps
16/07/2020	3rd Emptying of traps	22/07/2021	3rd Emptying of traps
23/07/2020	4th Emptying of traps	29/07/2021	4th Emptying of traps Replacement of both nets and pheromones
30/07/2020	5th Emptying of traps Replacement of both nets and pheromones 1st transplant damage sampling	05/08/2021	5th Emptying of traps
06/08/2020	6th Emptying of traps 2nd transplant damage sampling	12/08/2021	6th Emptying of traps 1st transplant damage sampling
13/08/2020	7th Emptying of traps	19/08/2021	7th Emptying of traps 2nd transplant damage sampling
20/08/2020	8th Emptying of traps 3rd transplant damage sampling	26/09/2021	8th Emptying of traps Replacement of both nets and pheromones
27/08/2020	9th Emptying of traps Replacement of both nets and pheromones	02/09/2021	9th Emptying of traps
03/09/2020	10th Emptying of traps	09/09/2021	10th Emptying of traps 3rd transplant damage sampling
10/09/2020	11th Emptying of traps	16/09/2021	11th Emptying of traps
17/09/2020	12th Emptying of traps	23/09/2021	12th Emptying of traps
24/09/2020	13th Emptying and remotion of traps	30/09/2021	13th Emptying and remotion of traps

5.4. Statistical analysis

A) Trials on pheromones effectiveness and traps placement

L. rugulipennis specimens captured in the field and identified in the laboratory were divided by species and sex. Data were represented using a graph which showed the trend of *L. rugulipennis* catches (total number of catches at each emptying) obtained by different treatments under test, together with the sex ratio of catches.
In addition, a comparison between the three treatments was carried out performing factorial ANOVA (n=3), where pheromone (yes / no) and height (high / low) were the factors (considering subsequent transplants as randomized blocks). The analysis was followed by the Newman-Keuls post-hoc test ($P < 0.05$), to show any significant difference between treatments.

All the statistical analyses were carried out with IBM SPSS Statistics (ver. 26).

B) Semiochemical-assisted trap cropping

Also in the semiochemical-assisted trap cropping trial, captured insects were identified in the laboratory and specimens of *L. rugulipennis* were divided by sex and then counted. Data were represented as mean number (± SE) of *L. rugulipennis* specimens captured and relative sex-ratio, at each emptying, in order to create a graph showing the trend of catches.

Regarding the damage to lettuce, for each of the three transplants, healthy and damaged leaves on 25 lettuce heads / treatment were counted, diversifying the % of damage (= damaged leaves / total leaves * 100) on the head as it is at harvest (whole head) and the head clean and ready for marketing (commercial head). Subsequently, data were processed using a One-way ANOVA (n = 3), considering subsequent transplants as randomized blocks, in order to highlight any significant differences between the two treatments.

Sampling years were analysed separately.

All the statistical analyses were carried out with IBM SPSS Statistics (ver. 26).

5.5. Meteorological data

Meteorological data relating to the period in which both trials were carried out in the study areas are reported in Figg. 32 and 33:

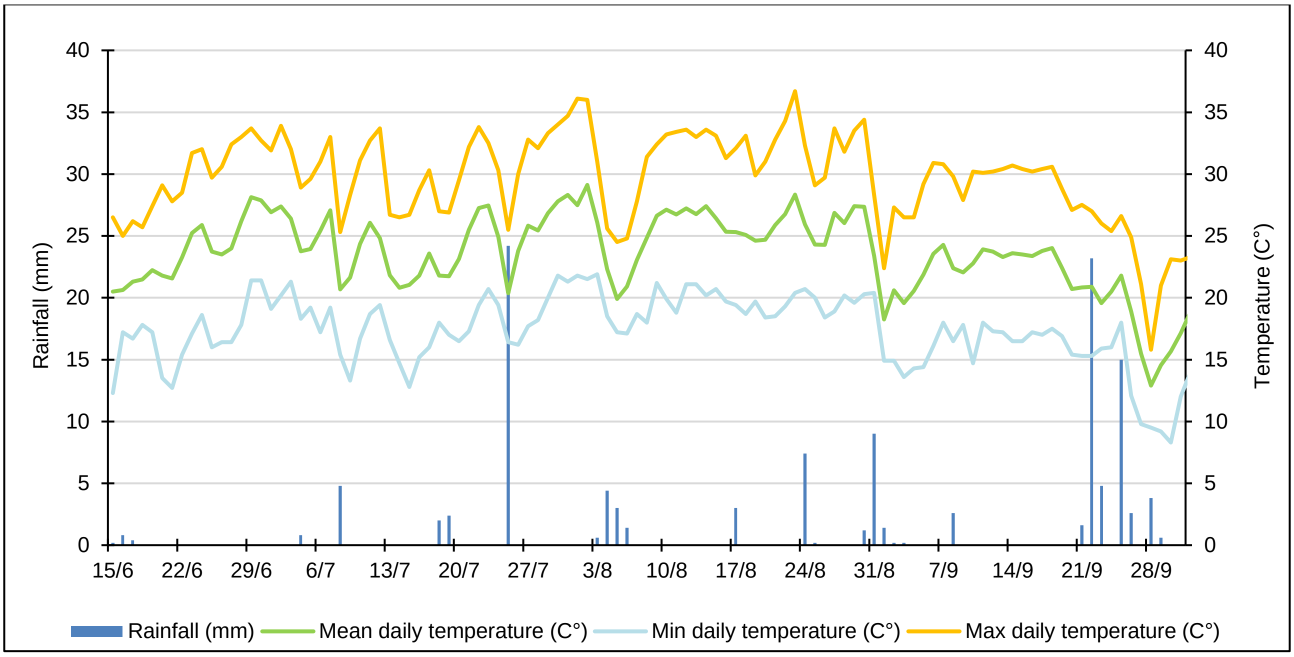

Figure 32 - Meteorological data, growing season 2020.

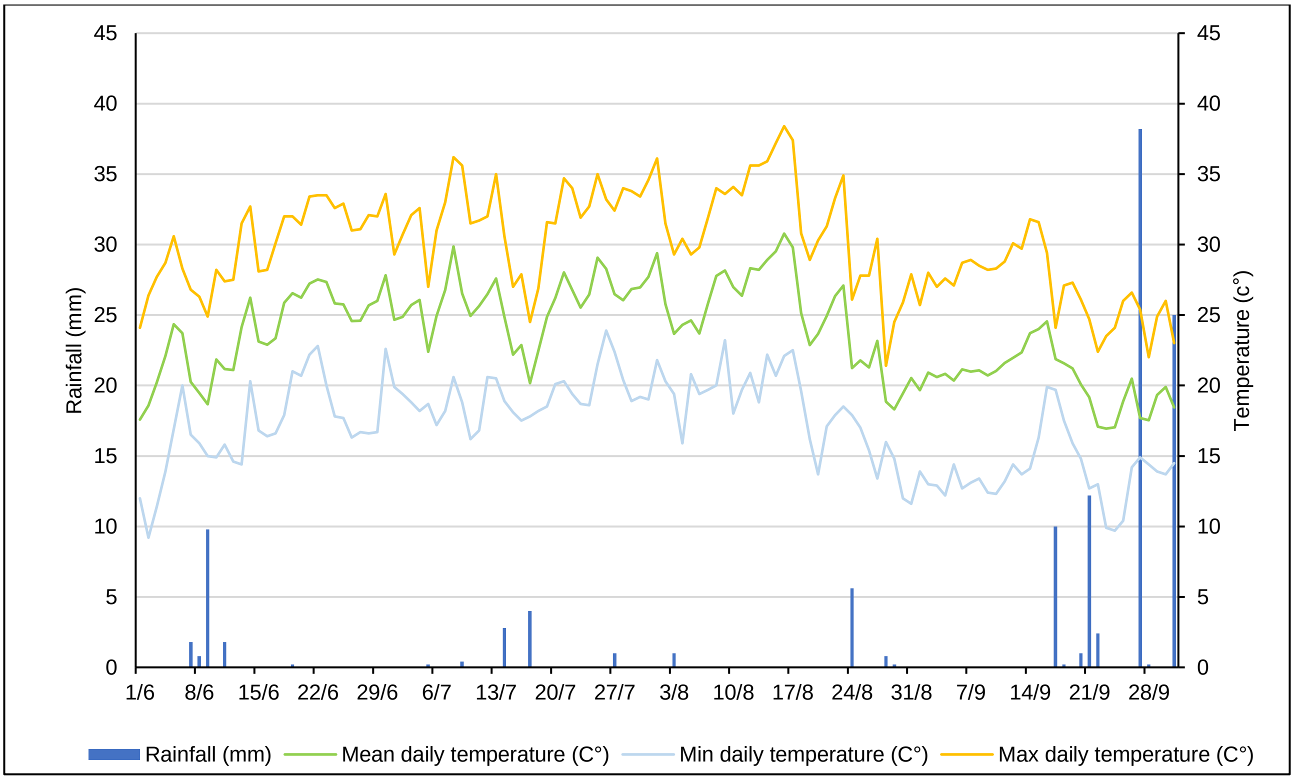

Figure 33 - Meteorological data, growing season 2021.

6. *RESULTS*

6.1. Trap cropping technique against flea beetles on sugar beets

2020 growing season results

In 2020 growing season, in the first assessment, carried out on the BBCH12 stage of sugar beet, from the comparison between the mean damage indices (calculated as the average of the damage indexes of each couple of rows at each distance) in the control treatment plot and in the trap-crop treatment plot (Fig. 34), it is shown that the trap-crop allowed to contain the damage if compared to the Control plot. Indeed, all the farms showed higher damage indices in the rows sampled in the Control than in those sampled near the trap-crop border. In particular, in the farm Rossi, the mean damage index recorded in the control plot was 23.3 %, while the one calculated in the trap-crop plot was 9.0 %. Similarly, in the farm Badile, the average damage in the control plot was 19.6 %, while in the rows near the trap-crop was 12.4 %. In the farm Delta, the mean damage index in the control plot reached 29.0 %, while near the trap-crop 21.2 %. Finally, in the farm Bergonzini, the average damage index in the control plot recorded the highest data of the assessment, with 31.9 %; on the contrary, the damage index near the trap-crop border was 13.8 %.

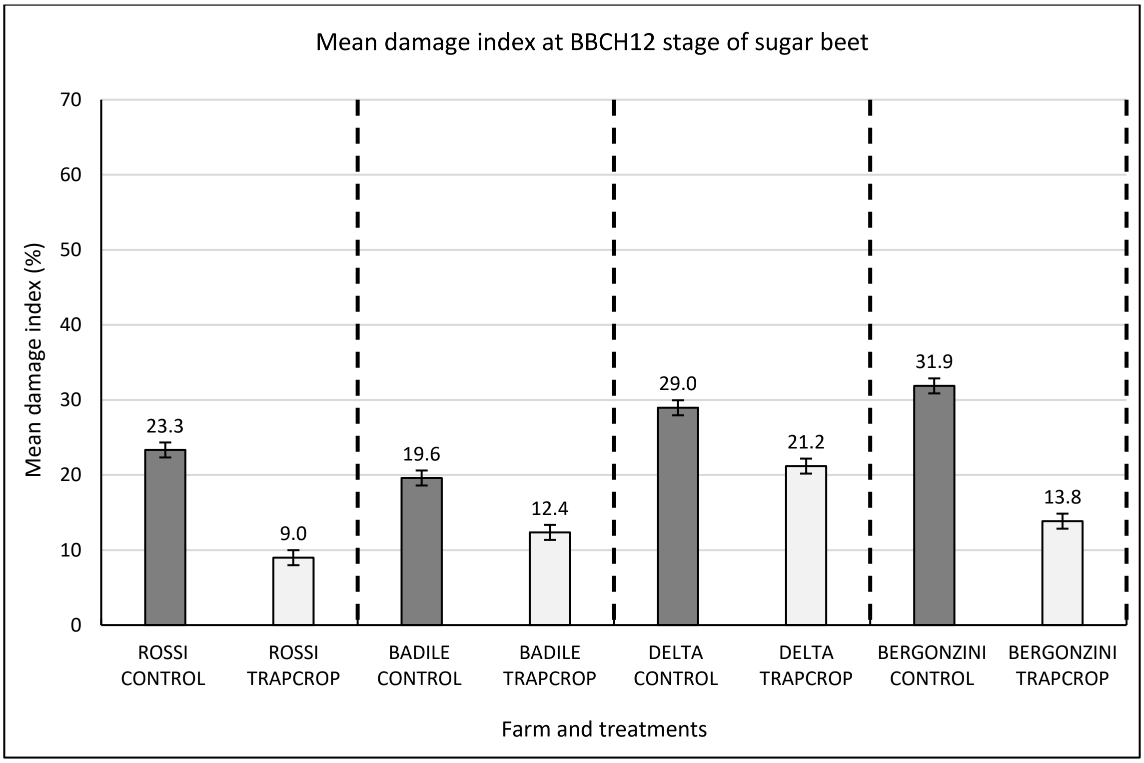

Figure 34 - Mean damage indices recorded BBCH12 stage of sugar beets in the four farms, 2020 growing season.

In the second assessment, carried out on the BBCH14 stage of sugar beet, from the comparison between the two treatment's average damage indices (Fig. 35), a generalized increase in flea beetle damage in all farms, compared to that of first assessment was shown. Despite the increased levels of injury, trap-crop treatment has still shown good efficacy in containing flea beetles damage, compared to the control plot. Indeed, in the farm Rossi, a mean damage index of 40.4 % was highlighted in the control plot, while in trap-crop plot the damage index was 22.1 %. In the farm Badile, the mean damage index in the control plot was 40. 9%, while in the trap-crop plot was 21.0 %. In the farm Delta the control treatment's damage index reached 49.4 %, while in trap-crop treatment was 35.2 %. In the farm Bergonzini the mean damage index in the control plot recorded 62.2 %., while it was 30.0 % in the trap-crop treatment.

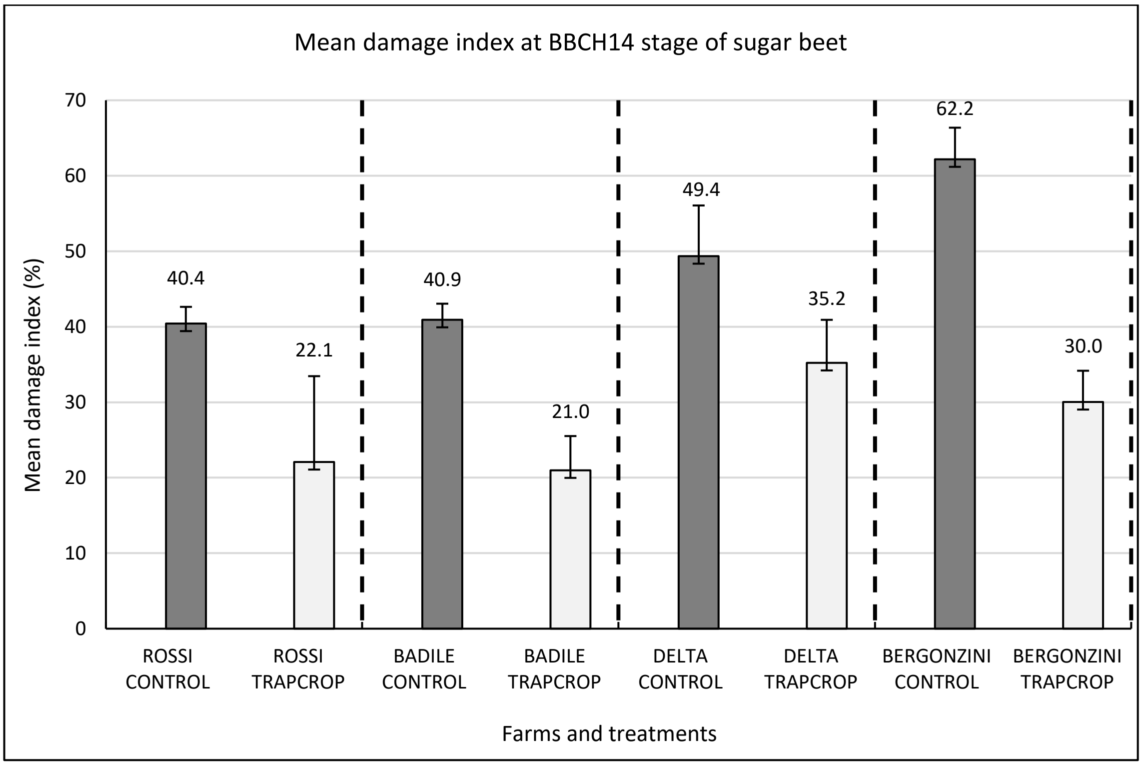

Figure 35 - Mean damage indices recorded BBCH14 stage of sugar beets in the four farms, 2020 growing season.

Even in the assessment carried out on the BBCH16 stage of sugar beets, comparing the mean damage indices of the control and trap-crop treatments (Fig. 36), a reduction of the damage on the sugar beet was highlighted in the rows close to the trap-crop compared to the rows of the control plot. In particular, in the farm Rossi, compared to a mean damage index recorded in the control plot of 19.6 %, in the trap-crop plot the damage index was 15.6 %. In the farm Badile, the damage index in the control plot was 30.8 %, while in the trap-crop plot it was 12.2 %. As for the farm Delta, the control plot recorded a damage index of 27.3 %, while in the trap-crop plot was 20.1 %. Finally, in the farm Bergonzini, in the control plot a damage index of 42.6 % was highlighted, while in the trap-crop plot an effective limitation of the damage was shown, with a recorded damage index of 18.1%.

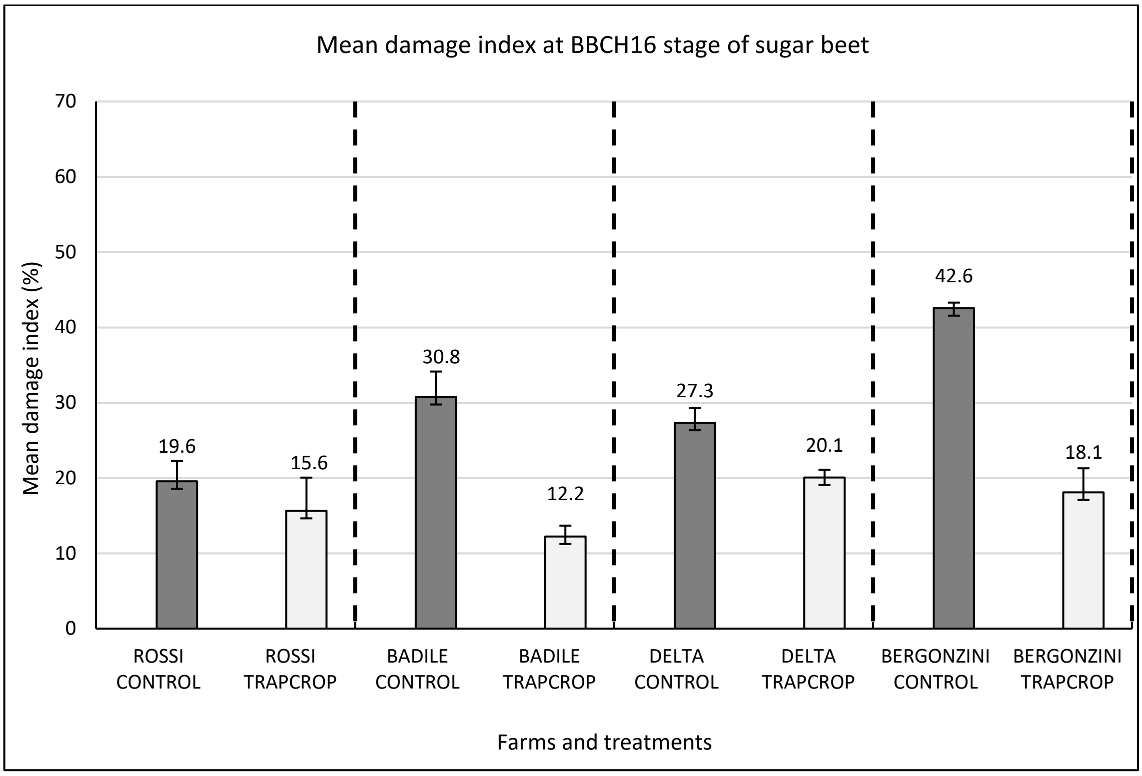

Figure 36 - Mean damage indices recorded BBCH16 stage of sugar beets in the four farms, 2020 growing season.

2021 growing season results

In the first assessment, carried out at BBCH12 stage of sugar beet, the comparison between the mean damage indices in the control treatment plot and in the trap-crop treatment plot (Fig. 37) highlighted that the trap-crop permitted a certain containment of the damage compared to the control plot. In particular, in the farm Rossi, although the presence of a modest flea beetle infestation in the trial field, the mean damage index calculated in the control plot was 9.7 %, while that calculated in the trap-crop plot was 6.0 %. In the farm Badile, where instead a strong attack was underway, in the control plot a mean damage index of 80.4 % was recorded, while that of the trap-crop treatment plot was 40.5 %. In the farm Delta the mean damage index in the control plot reached 26.5 %, while in the trap-crop plot was 22.6 %. Finally, in the farm Bergonzini, the mean damage index in the control treatment plot recorded 31.5 %, while in the trap-crop treatment plot it was 14.0 %.

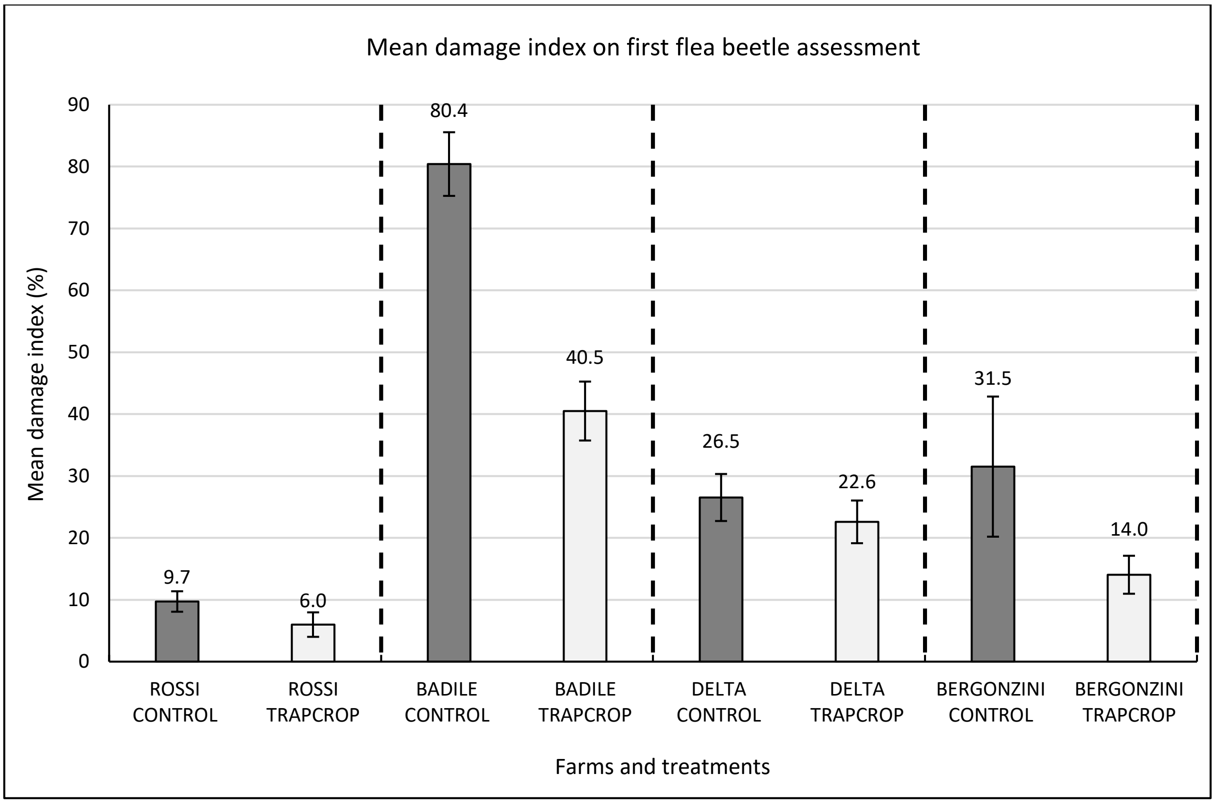

Figure 37 - Mean damage indices recorded at BBCH12 stage of sugar beets in the four farms, 2021 growing season.

Also in the second assessment, carried out at BBCH14 stage of sugar beet, from the comparison between the average damage indices of the two treatments (Fig. 38), a containment effect of flea beetle's infestations due to the trap-crop was showed again. Indeed, in the farm Rossi, an average damage index in the control treatment of 13.9 % was highlighted, while in the trap-crop treatment the damage index was 7.5 %. In the farm Badile, a damage index of 84.3 % was recorded in the control plot, while in the trap-crop was 46.2 %. In the farm Delta, the mean damage index calculated in the control treatment plot was 29.3 %, while that of trap-crop plot was 26.9 %. In the farm Bergonzini, an average damage index of 44.2 % was recorded in the control plot, while in the trap-crop plot it recorded 35.0 %.

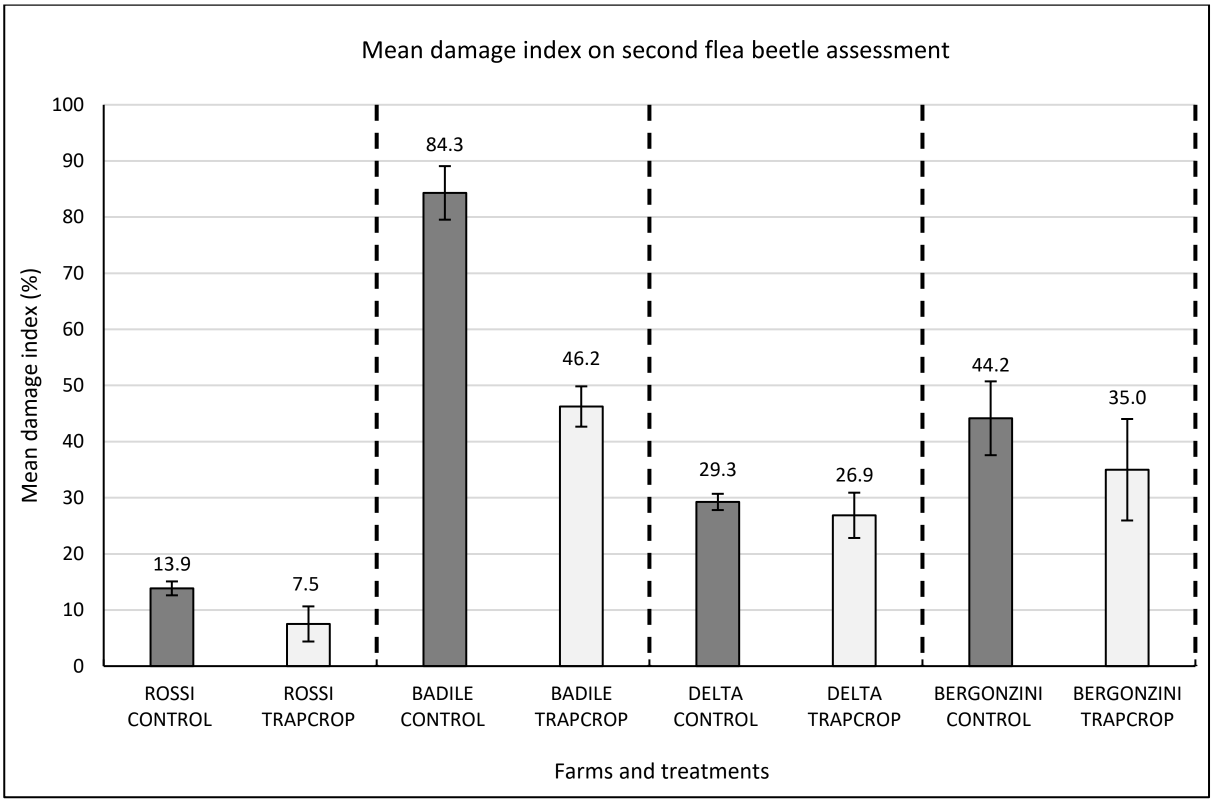

Figure 38 - Mean damage indices recorded at BBCh14 stage of sugar beets in the four farms, 2021 growing season.

In the third assessment, carried out at BBCH16 stage of sugar beet, the comparison between the average damage indices in the control and trap-crop treatments (Fig. 39), highlighted how, also in this case, in all farms, the trap-crop limited flea beetle's damage in the neighbouring sugar beet rows compared to those of the control treatment. In particular, in the farm Rossi, an average damage index of 18.2 % was recorded in the control treatment plot, while in the trap-crop plot this was 7.1 %. In the Badile farm, the average damage index in the control plot was 76.2 %, while in the trap-crop treatment plot the damage was contained at 62.6 %. In the farm Delta, the control plot highlighted a damage index of 32.0 %, while that recorded in the trap-crop treatment it was 24.6 %. Finally, in the farm Bergonzini, the control plot recorded a damage index of 37.3 %, while the damage index of the trap-crop treatment was 28.8 %.

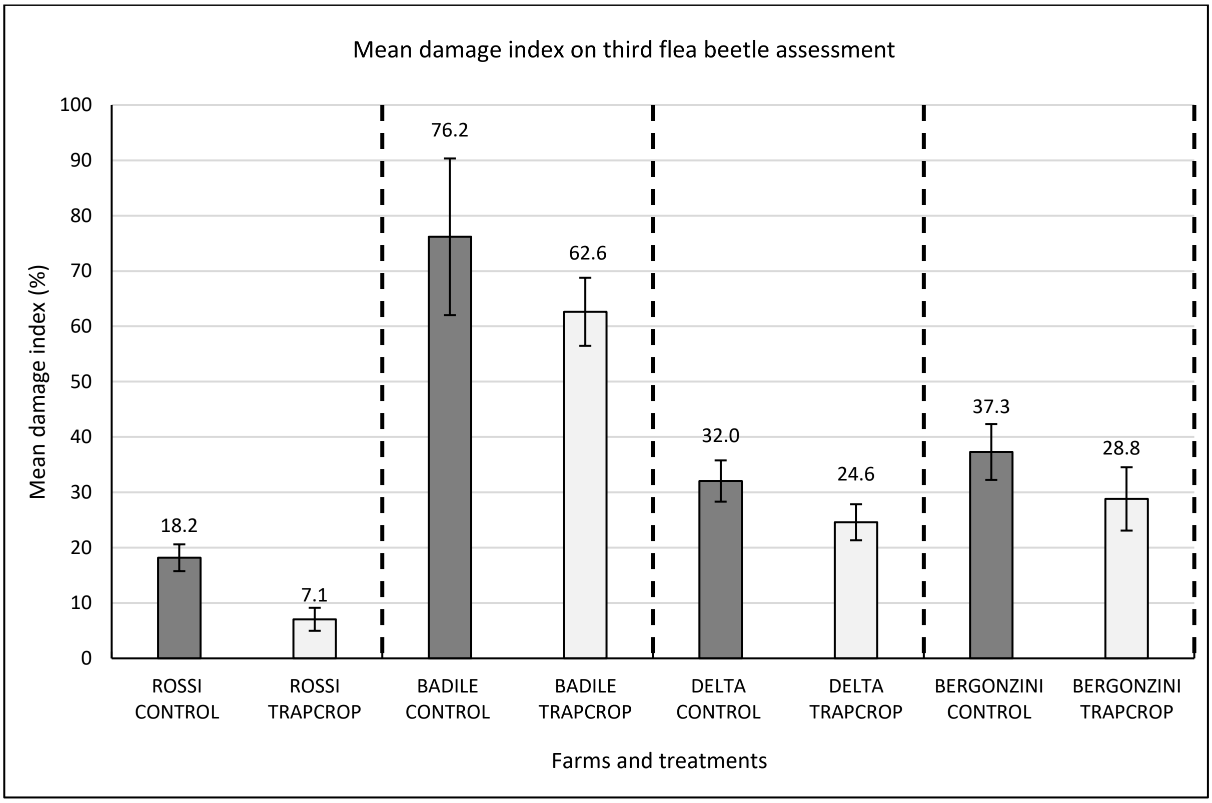

Figure 39 - Mean damage indices recorded at BBCH16 stage of sugar beets in the four farms, 2021 growing season.

Results of both growing seasons pooled

In both growing seasons, similar trends of damage index caused by flea beetles were detected. In 2020, mean damage index decreased from 34.74% (CI 95% = 30.69–38.79%) on sugar beet growing at the bare soil edge of the plots to 19.23% (CI 95% = 16.11–22.34%) of the plants with a trap crop border with an overall reduction of 44.66%. In 2021, mean damage index decreased from 40.29% (CI 95% = 32.50–48.07%) on sugar beet grown at the bare soil borders to 26.82% (CI 95% = 21.56–32.08%) of the plant rows with a trap crop border with an overall reduction of 33.44%.

Pooling the two growing seasons, GLMM detected significant effects of treatments, distances from border and BBCH stages of sugar beet plants on the index of damage due to flea beetles ($P < 0.001$) (Table 4). Moreover, the damage index was also significantly affected by the interaction of treatment x distance ($P < 0.001$) (Fig. 40). In trap crop rows, damage significantly decreased at increasing distances from field border, whereas damage did not vary with distance in control plots. Damage index in trap crop treatment at 2 m from the border did not show any significant differences in comparison with controls ($P > 0.05$), whereas lower damage was detected in trap crop rows than in controls at 6, 12 and 15 m from the borders ($P < 0.001$).

The lowest damage index was found at BBCH12, and the highest index was found at BBCH14, with intermediate damage at BBCH stage 16 ($P < 0.001$) (Fig. 41). Given that none of the interactions including BBCH stage of sugar beet were significant, the effects of treatment and distance on damage by flea beetles did not vary with phenological growth stage.

Table 4 - Results of the GLMM testing the effect of treatment, distance, BBCH stage of sugar beet and their interactions on flea beetle damage index. Data of both growing seasons were considered together.

Factor or Interaction	**F**	**d.f.1**	**d.f.2**	***P***
Treatment (Trap crop vs Control)	119.04	1	55.29	<0.001
Distances (2, 6, 12, 15 m)	10.69	3	55.29	<0.001
BBCH stage of sugar beet (12, 14, 16)	40.47	2	108.09	<0.001
Treatment * Distances	12.21	3	55.29	<0.001
Treatment * BBCH stage of sugar beet	0.22	2	108.09	.80
Distances * BBCH stage of sugar beet	0.70	6	112.72	.65
Treatment * Distances * BBCH stage of sugar beet	0.42	6	112.72	.86

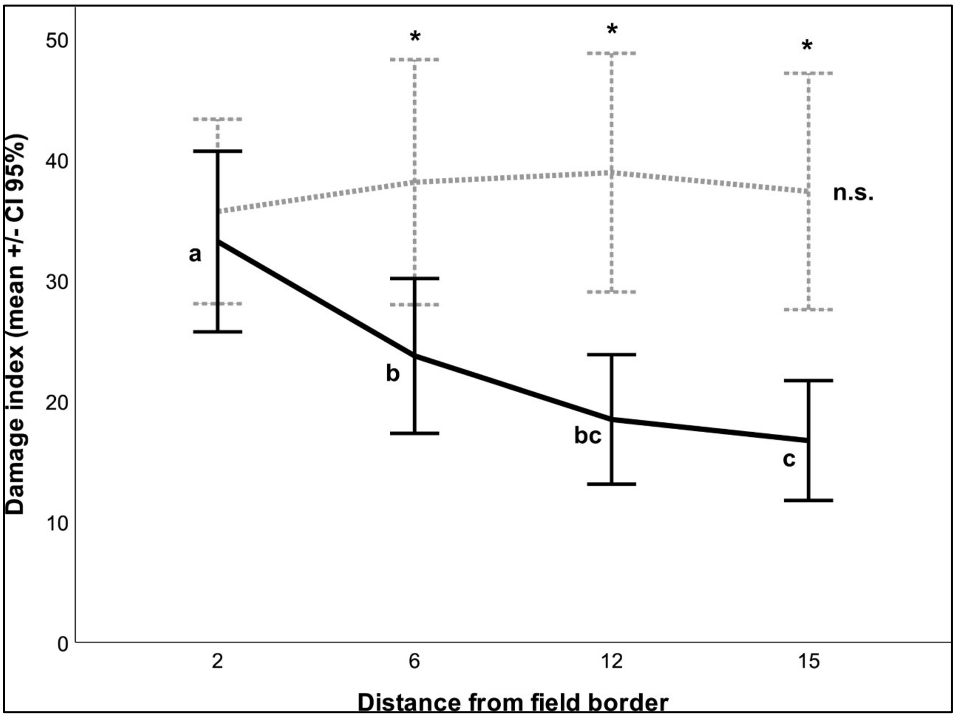

Figure 40 - Damage index due to flea beetles in sugar beet rows in function of border type (trap crop represented by a black solid line vs bare soil, represented by a dotted grey line) and distances from the border. Asterisks denote significant differences between treatments within each distance. Different letters indicate significant differences of distances within each treatment as detected by a multiple comparison test with Bonferroni sequential adjustment (P < 0.05).

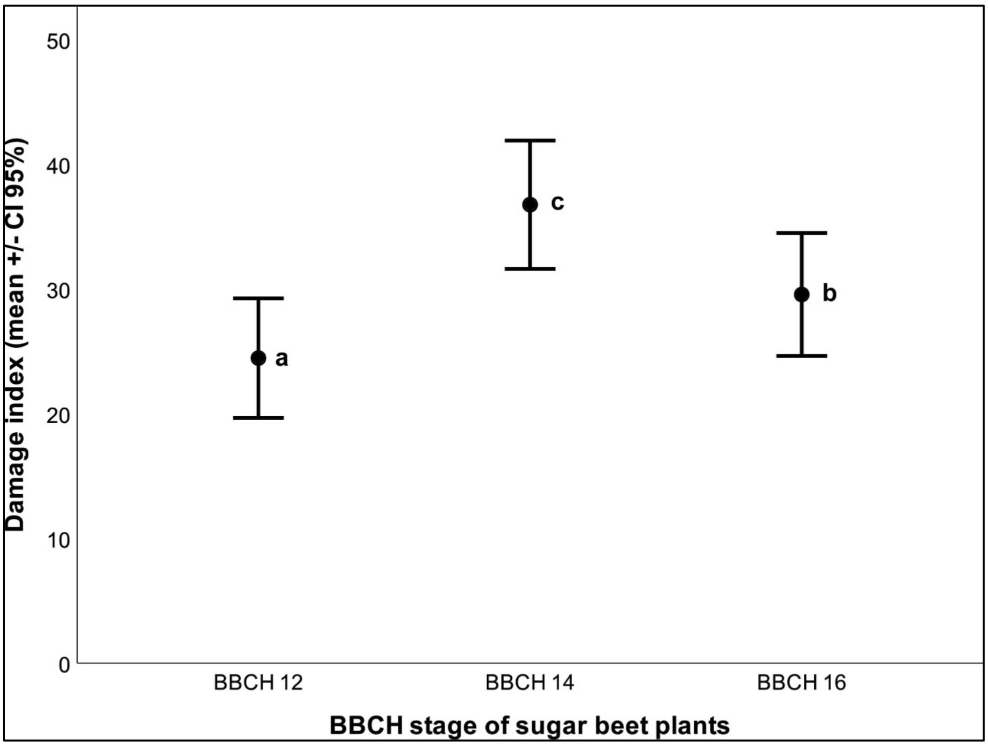

Figure 41 - Damage index due to flea beetles in sugar beet rows as a function of BBCH stage of sugar beet. Different letters indicate significant differences be- tween development stages as detected by a multiple comparison test with Bonferroni sequential adjustment (P < 0.05).

6.2. *Lygus rugulipennis* on lettuce

A) Trials on pheromones effectiveness and traps placement

Lygus rugulipennis catches of all treatment traps remained at modest levels until mid-July, growing rapidly in the following weeks (Fig. 42). However, catches were mainly concentrated in the month of August in all treatment traps, while in the month of September very low catch levels were recorded (Fig. 43-46).
In 2021 growing season has been highlighted that, in both baited and unbaited treatments, traps permitted to catch both *L. rugulipennis* sexes.

Throughout the trial, a total of 162 *L. rugulipennis* adults were captured in baited low traps while 113 specimens in empty low traps. In baited high traps *L. rugulipennis* catches were 84, while in empty high traps the specimens caught were only 61 (Fig. 47). In baited traps, captured male specimens proved to be prevalent over females, while in empty traps the sex ratio of captured *L. rugulipennis* specimens was almost equal or slightly in favour of females.

Both baited and empty traps confirmed their attraction towards Coccinellidae beetles, especially of *H. variegata* and, to a lesser extent, *H. axyridis*, *C. septempunctata*, and *P. quatordecimpunctata*. Traps positioned at ground level recorded the lowest number of total Coccinellidae catches, while the other two treatment traps, both placed at the hight of 70 cm, showed almost double catch levels (Fig. 48).

The statistical analysis, carried out by performing a factorial ANOVA (n=3), where pheromone (yes/no) and height (high/low) were the factors, provided the following results:

- the catches of *L. rugulipennis* males in baited traps were significantly higher than empty traps (Fig. 49).
- No significant differences were found in the catches of female *L. rugulipennis* between baited and empty treatment traps (Fig. 50).
- The traps positioned at ground level recorded significantly higher catches of *L rugulipennis* specimens of both sexes than those positioned at a height of 70 cm (Fig. 51 and 52).
- No significant differences were found in the overall catches (males + females) of *L. rugulipennis* between baited and empty traps (Fig. 53).

- The traps positioned at ground level (Low) recorded significantly higher overall catches (males + females) of *L. rugulipennis* than those positioned at a height of 70 cm (High) (Fig. 54).

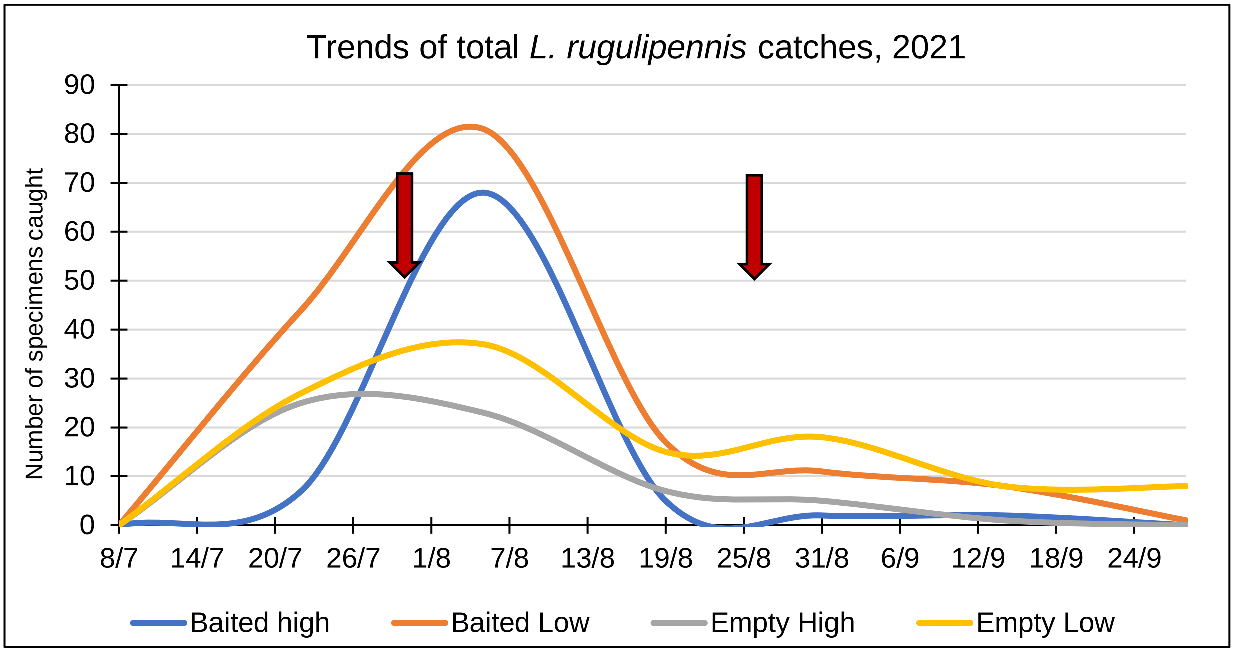

Figure 42 - Trends of total L. rugulipennis catches recorded by the different treatments, 2021 growing season. Red arrows indicate the dates of the substitution of the pheromones in all traps.

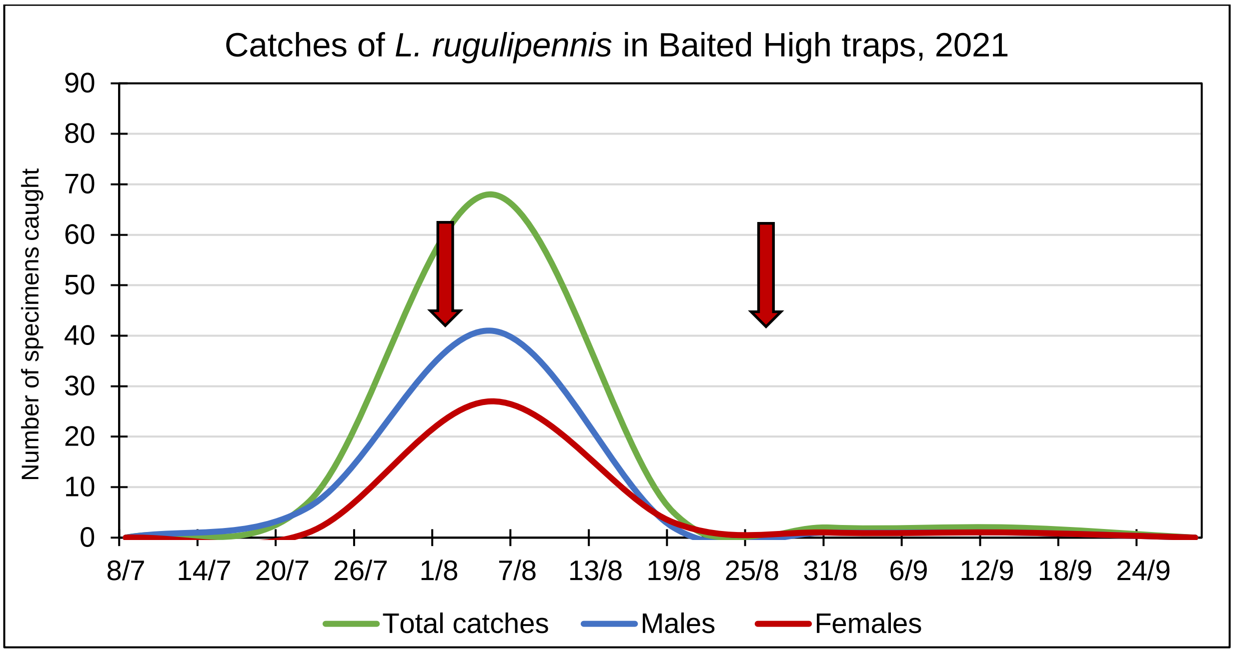

Figure 43 - Trends of L. rugulipennis catches recorded in baited high treatment, 2021 growing season. Red arrows indicate the dates of the substitution of the pheromones in all traps.

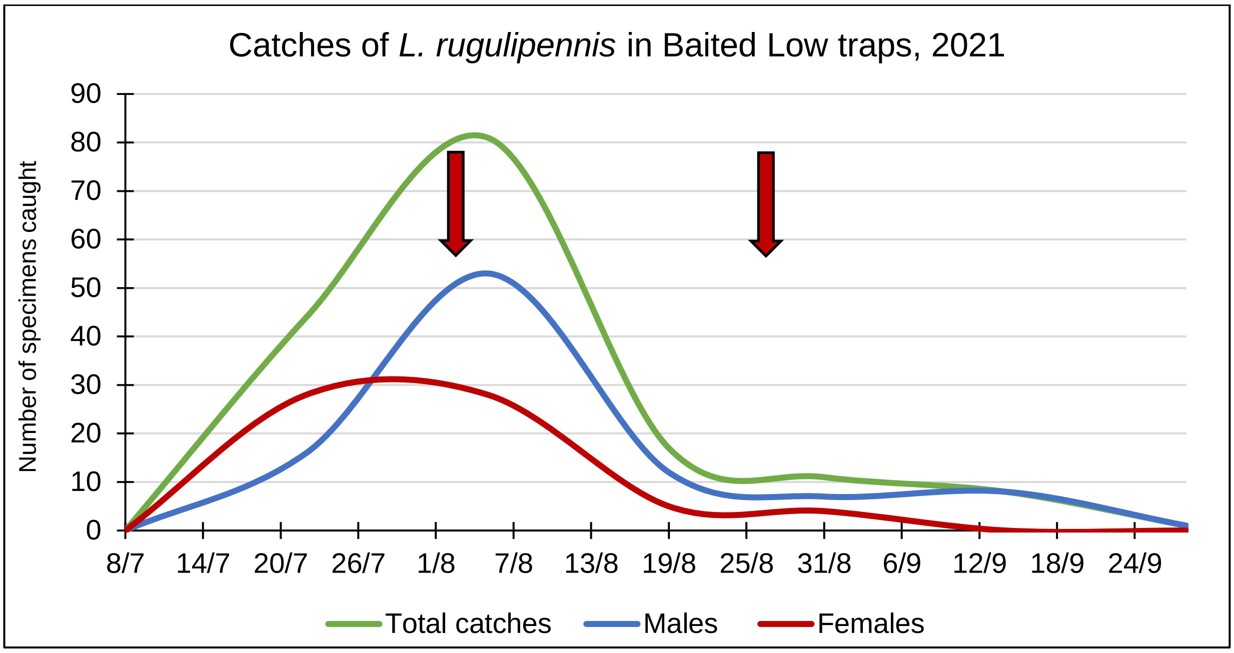

Figure 44 - Trends of L. rugulipennis catches recorded in baited low treatment, 2021 growing season. Red arrows indicate the dates of the substitution of the pheromones in all traps.

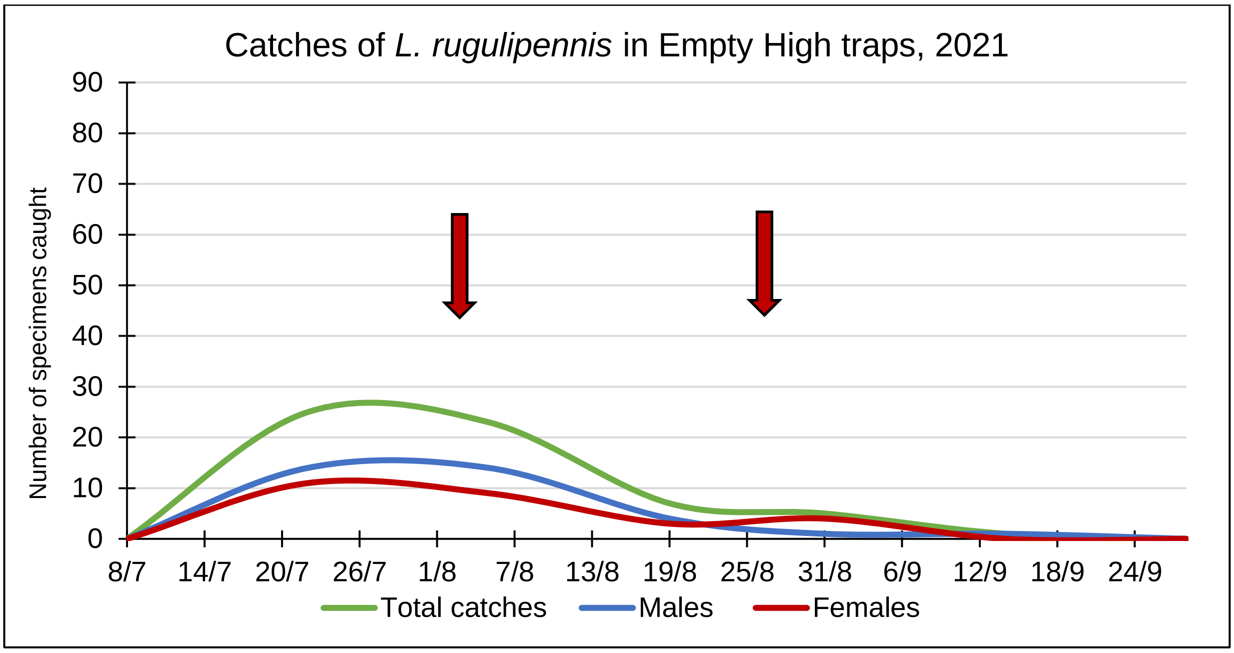

Figure 45 - Trends of L. rugulipennis catches recorded in empty high treatment, 2021 growing season. Red arrows indicate the dates of the substitution of the pheromones in all traps.

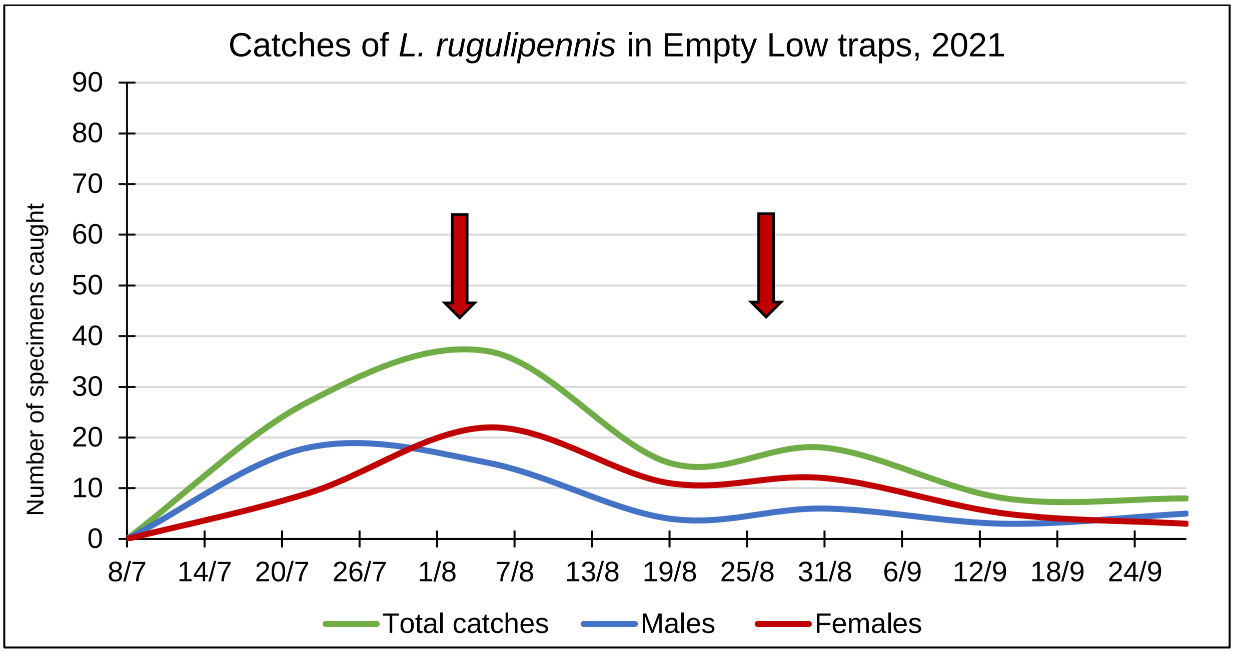

Figure 46 - Trends of L. rugulipennis catches recorded in empty low treatment, 2021 growing season. Red arrows indicate the dates of the substitution of the pheromones in all traps.

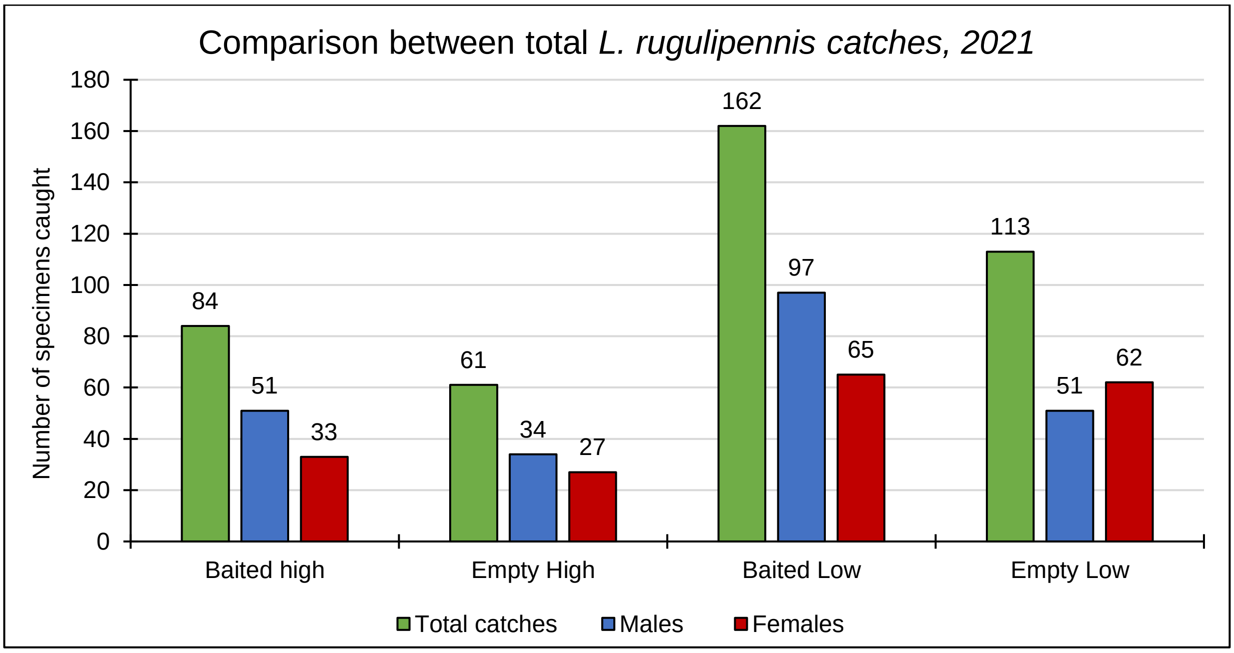

Figure 47 - Total L. rugulipennis catches recorded in different treatments, 2020 growing season.

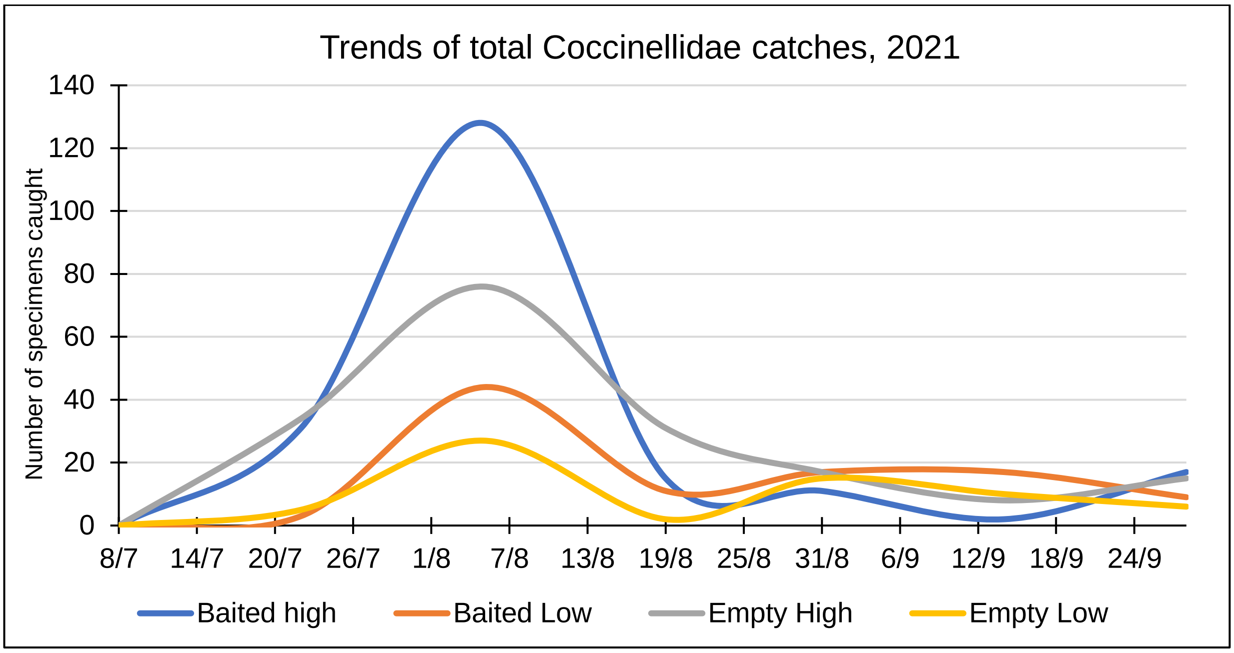

Figure 48 - Trends of total Coccinellidae catches recorded in different treatments, 2021 growing season.

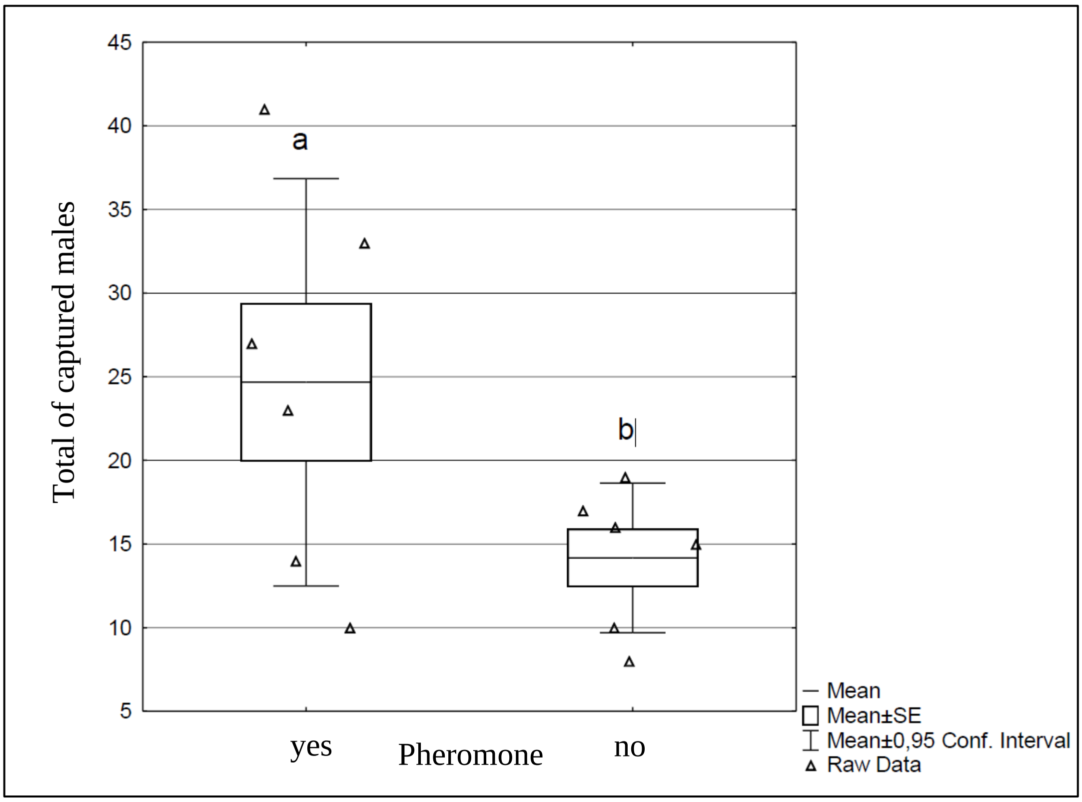

Figure 49 - Comparison of L. rugulipennis males catches in baited traps vs empty traps.

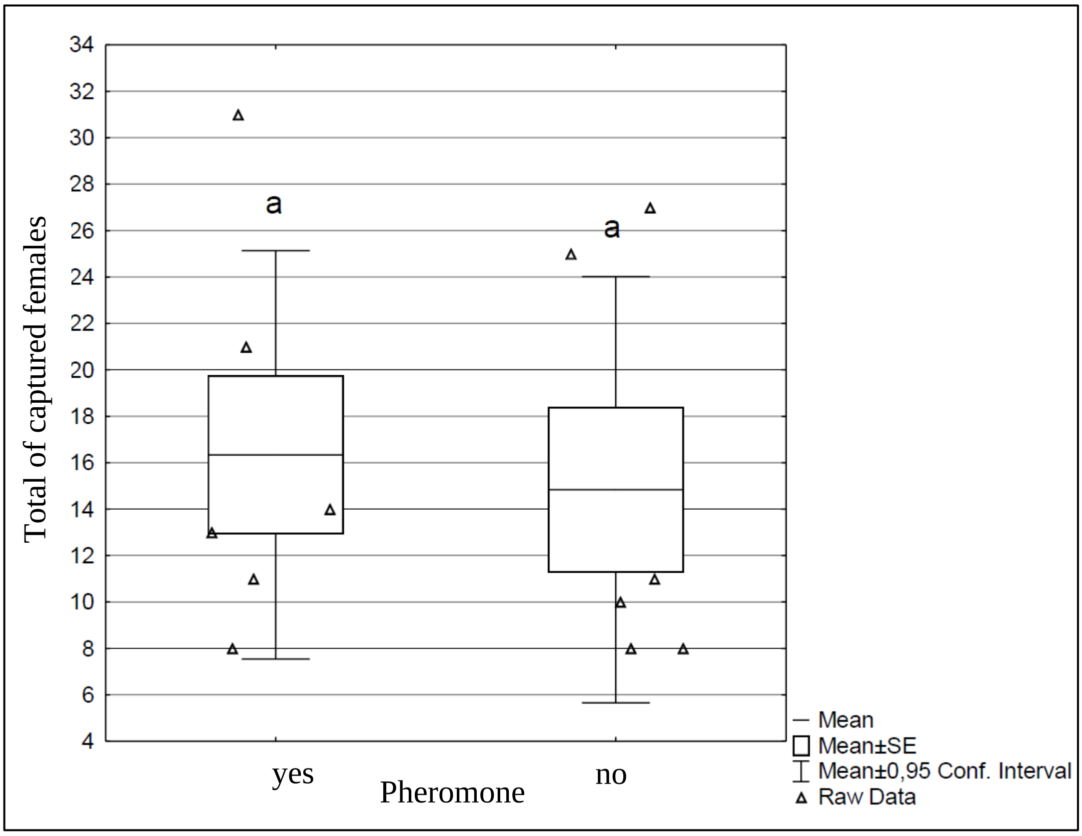

Figure 50 - Comparison of L. rugulipennis females catches in baited traps vs empty traps.

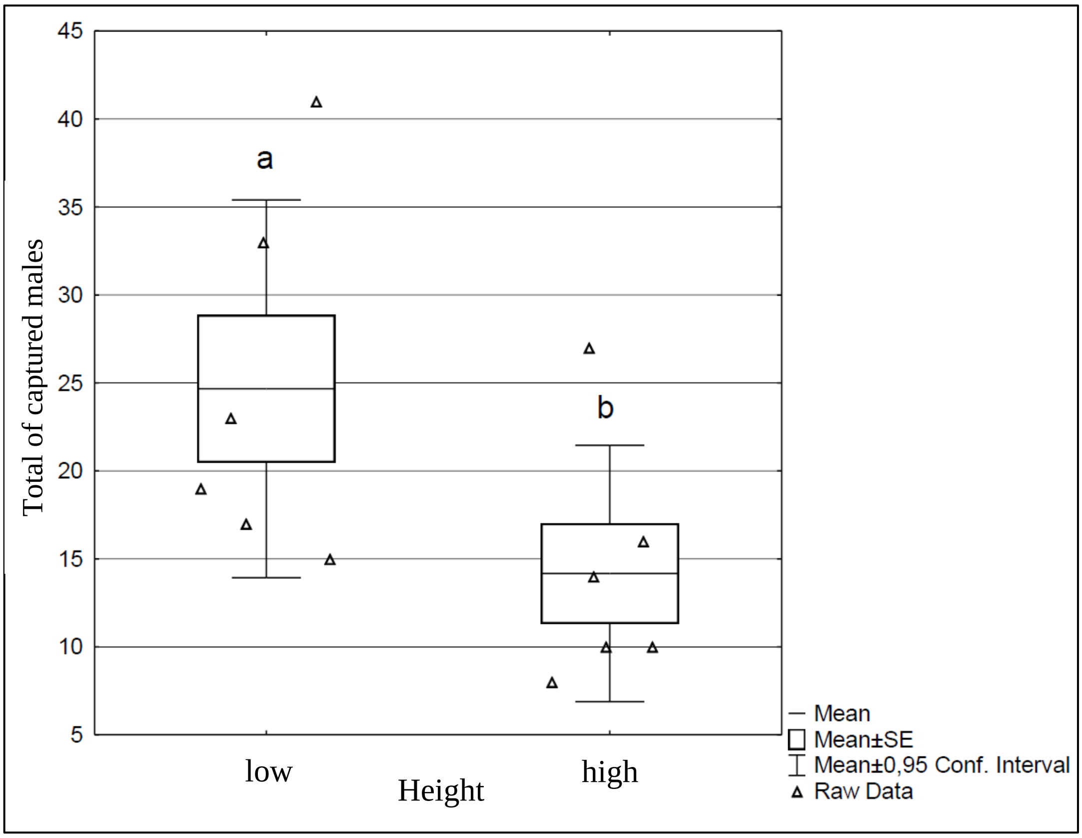

Figure 51 - Comparison of L. rugulipennis males catches in traps at different heights.

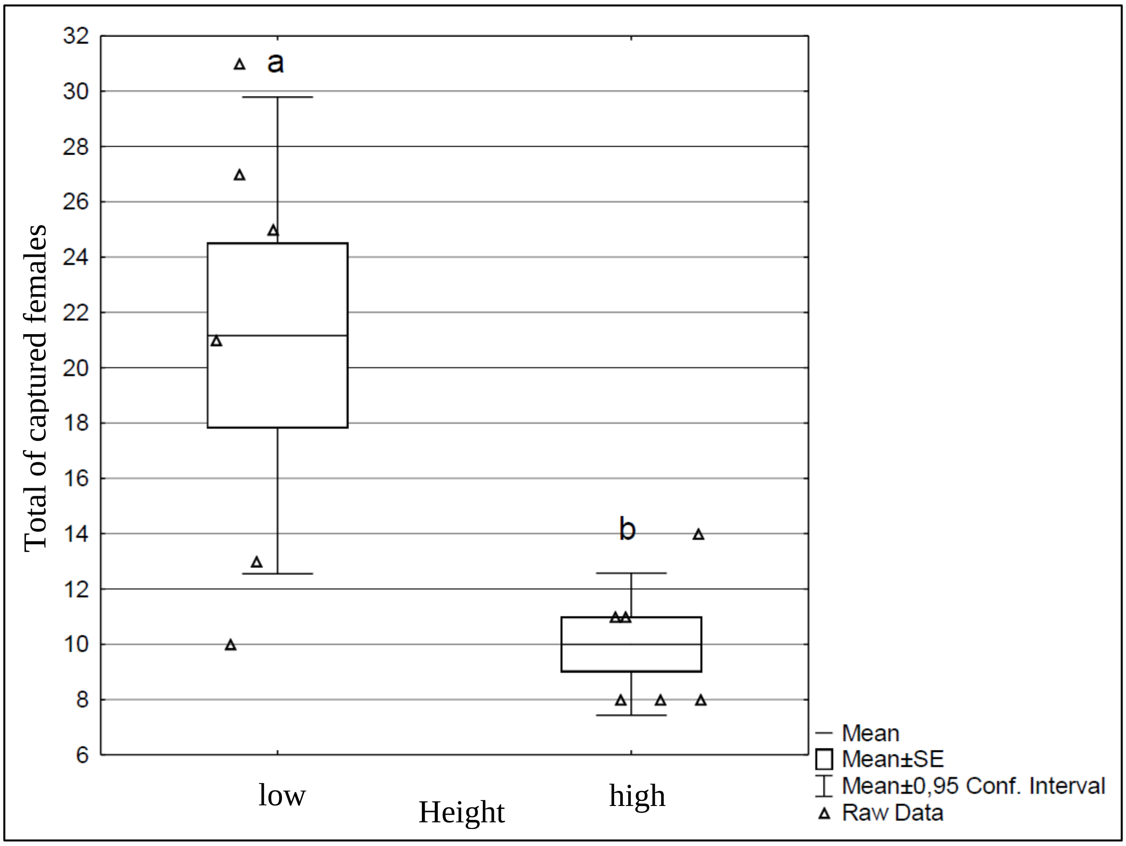

Figure 52 - Comparison of L. rugulipennis females catches in traps at different heights.

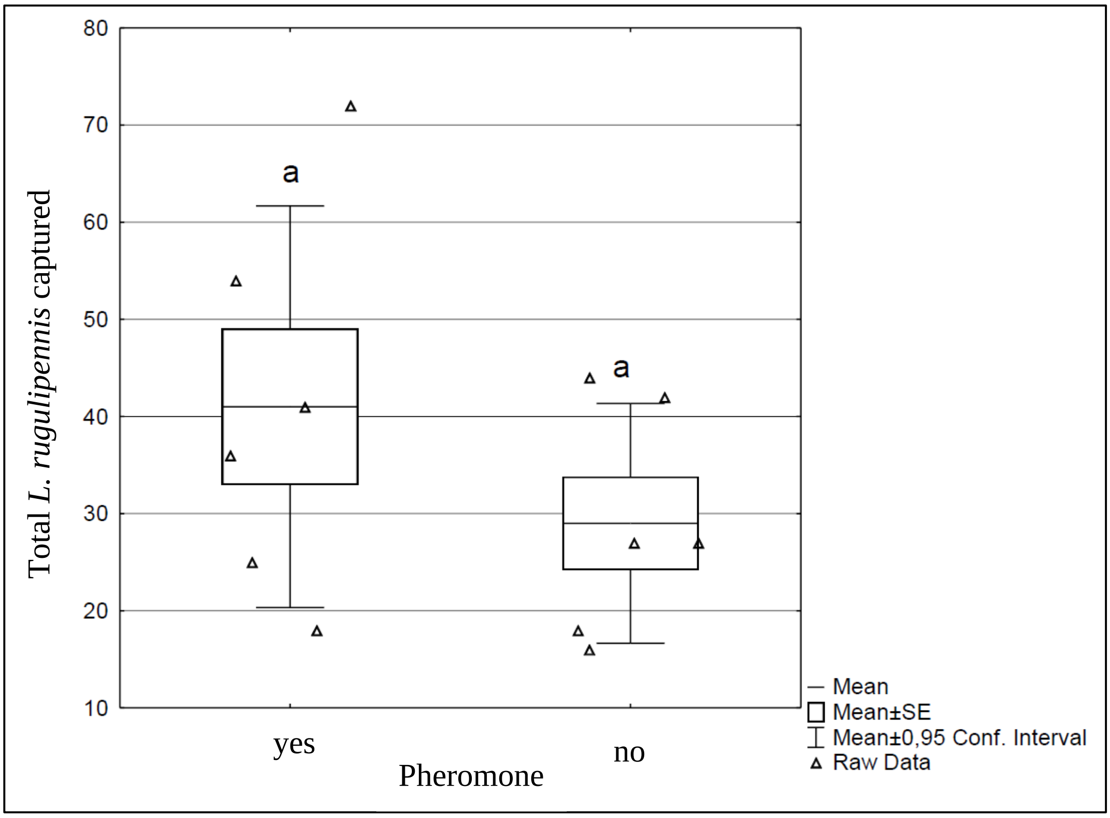

Figure 53 - Comparison of total L. rugulipennis catches in baited traps vs empty traps.

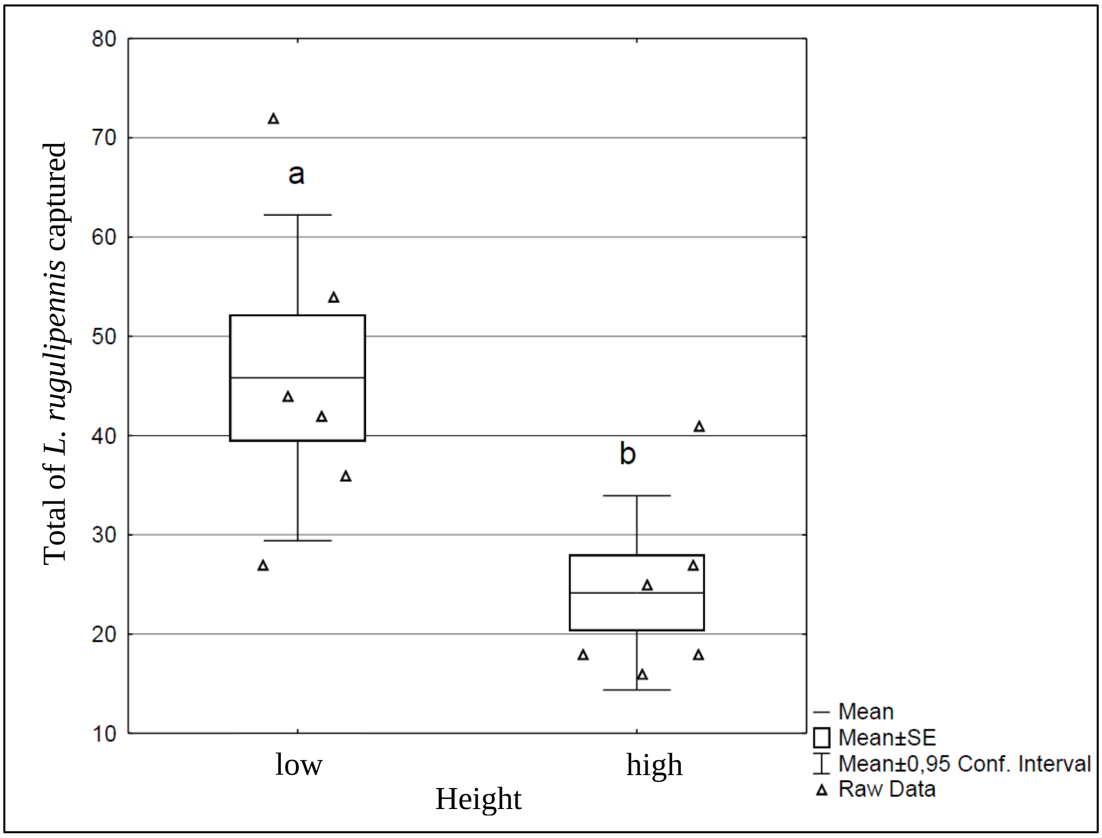

Figure 54 - Comparison of total L. rugulipennis catches in traps at different heights.

B) Semiochemical-assisted trap cropping

2020

In 2020 growing season, catches of *L. rugulipennis* in baited traps remained at modest levels until the half of July, subsequently recording two different peaks, the first of which of around 20 *L. rugulipennis*/trap was recorded at the beginning of the month of August, while a higher peak of 27 *L. rugulipennis*/trap occurred at end of the same month (Fig. 55). After that, the number of captured specimens steadily declined, reaching zero by the end of the month of September.

Pheromone baited traps showed an efficacy towards both sexes of *L. rugulipennis* with a sex-ratio of captured specimens always in favour of males, except for the last assessment (24/09/2020).

Regarding the damage samplings, the semiochemical-assisted trap cropping strategy based on the combined effect of the trap crop and the pheromone traps permitted to reduce the percentage of leaves damaged by *L. rugulipennis* considering both “whole lettuce heads” and “commercial lettuce heads” (Table 5).
In particular, a significant difference in the percentage of damaged leaves between control treatment and “trap crop + pheromone” treatment, only considering the commercial lettuce heads, was highlighted. Indeed, the reduction in the percentage of damage compared to the Control was 41.8 % (Fig. 57).
Conversely, no significant differences between the percentage of damaged leaves on the whole lettuce head, comparing control and semiochemical-assisted trap cropping treatments were highlighted. Indeed, on whole lettuce heads, the damage reduction was only 16.8% in favour of the semiochemical-assisted trap cropping strategy (Fig. 56).

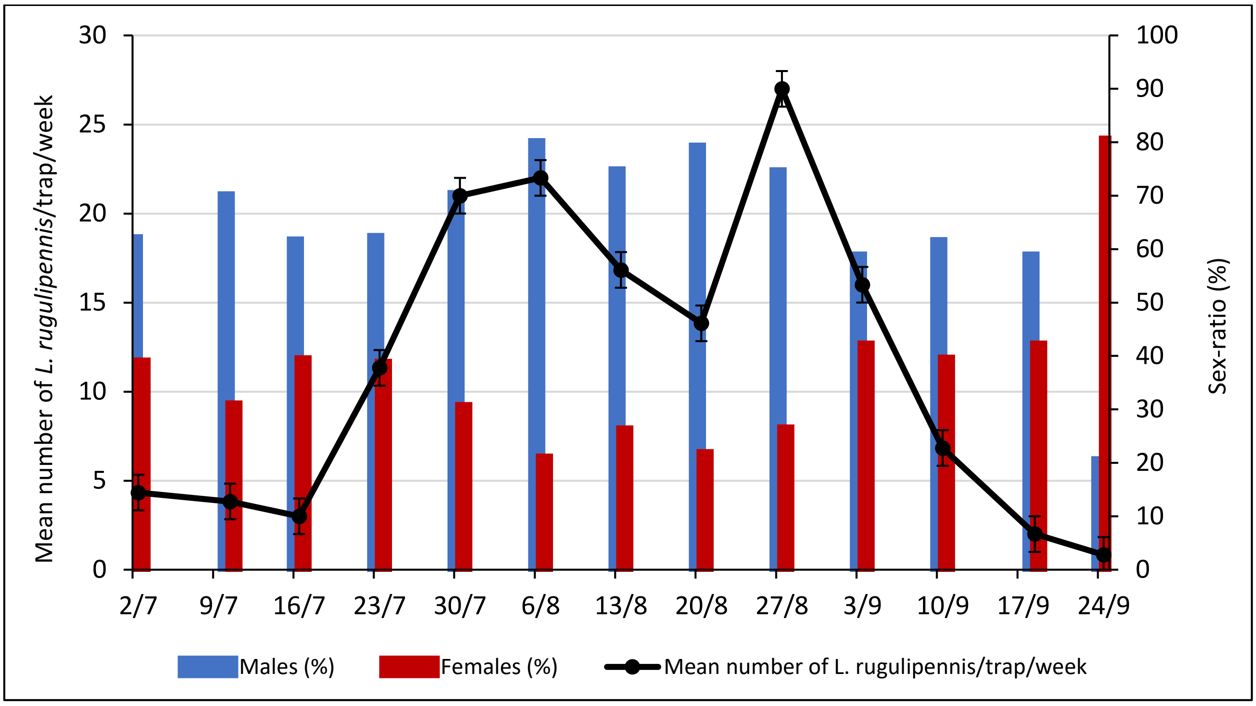

Figure 55 - Mean number (± SE) of L. rugulipennis specimens captured and relative sex-ratio, 2020 growing season.

Table 5 - L. rugulipennis damage (%) on lettuce heads in 2020 and 2021 growing seasons.

	Growing season			
	2020		2021	
	Whole lettuce head	Commercial lettuce head	Whole lettuce head	Commercial lettuce head
Control (mean ± ES)	39.1 ± 6.0 a	34.0 ± 4.9 a	55.3 ± 12.9 a	50.4 ± 13.9 a
Trap crop + Pheromone traps (mean ± ES)	32.6 ± 1.9 a	19.8 ± 1.5 b	38.6 ± 9.9 a	34.0 ± 10.0 a
Damage reduction (%)	16.8 %	41.8 %	30.2 %	32.6 %

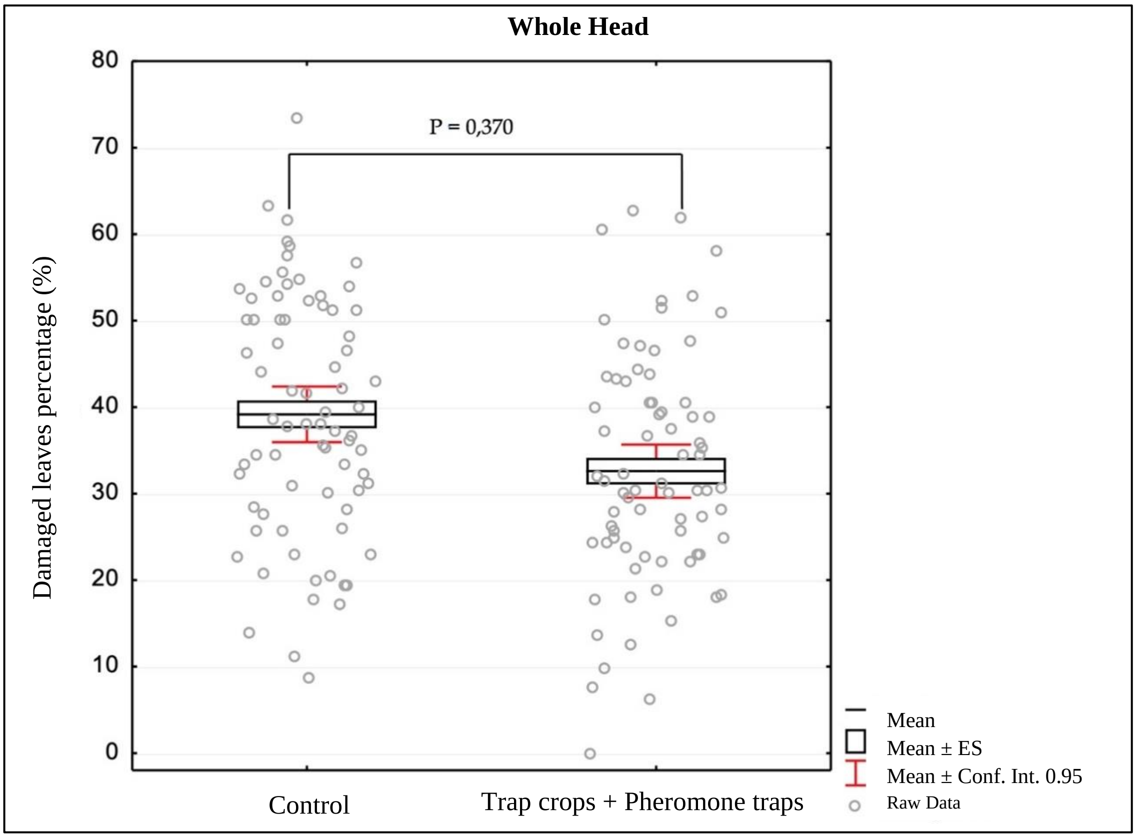

Figure 56 - Percentage of leaves damaged by L. rugulipennis in whole lettuce heads, 2020 growing season.

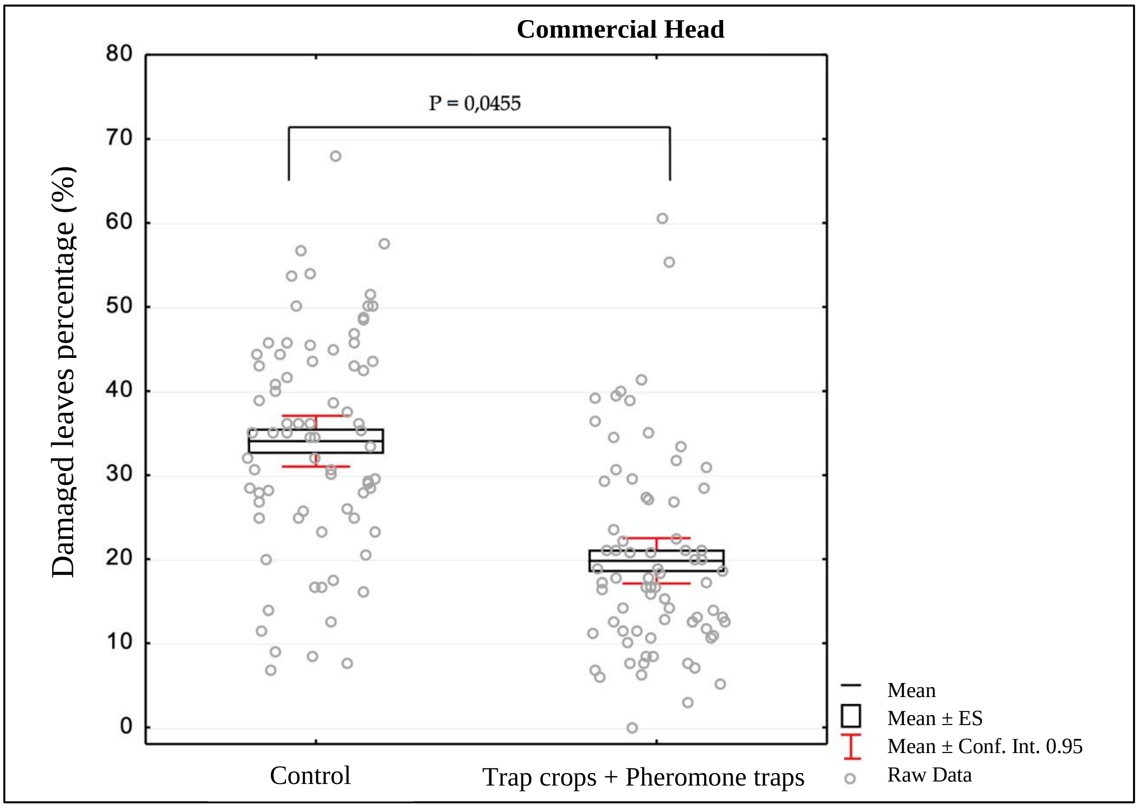

Figure 57 - Percentage of leaves damaged by L. rugulipennis in commercial lettuce heads, 2020 growing season.

2021

Compared to 2020, in 2021 growing season, *L. rugulipennis* in baited traps highlighted lower levels of catches throughout the entire period of the experiment, never reaching 10 specimens caught/trap/week. Trends of the catches showed two different peaks of around 7 *L. rugulipennis* caught/trap/week, the first of which in the middle of the month of August, and a second one at end of the month of September, just before the end of the experiment (Fig. 58).

As in 2020, also in 2021 pheromone baited traps showed an efficacy towards both sexes of *L. rugulipennis*. The sex-ratio of captured specimens highlighted a distinct prevalence of males over female, except for the first two samplings of the month of September (02/09 and 09/09), where males and females catches were equal.

Contrarywise 2020, in 2021 growing season no significant differences between the percentage of damaged leaves on both “whole lettuce heads” and “commercial lettuce heads” were highlighted, comparing control and the semiochemical-assisted trap cropping treatments (Fig. 59 and 60). Anyway, in the latter treatment, the reduction in the percentage of damage compared to the control was 30.2 % and 32.6 %, considering “whole lettuce heads” and “commercial lettuce heads”, respectively (Table 5).

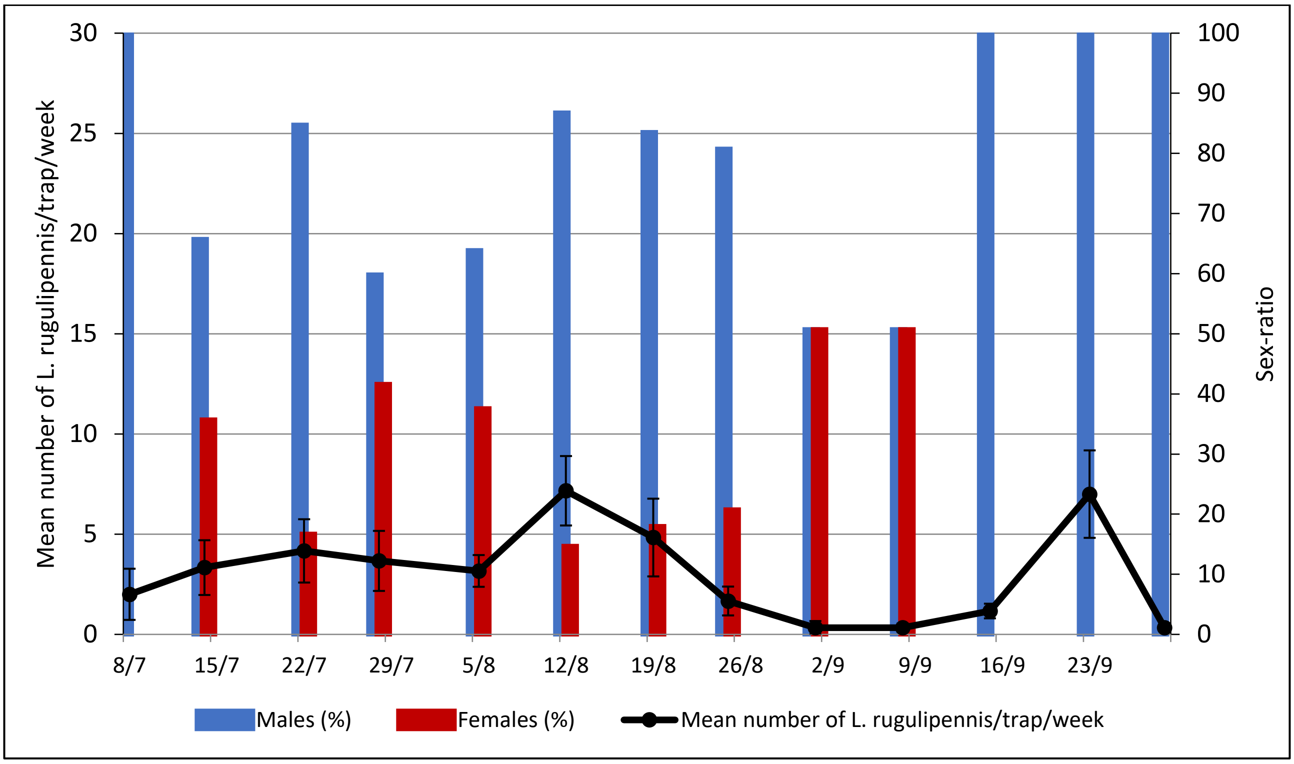

Figure 58 - Mean number (± SE) of L. rugulipennis specimens captured and relative sex-ratio, 2021 growing season.

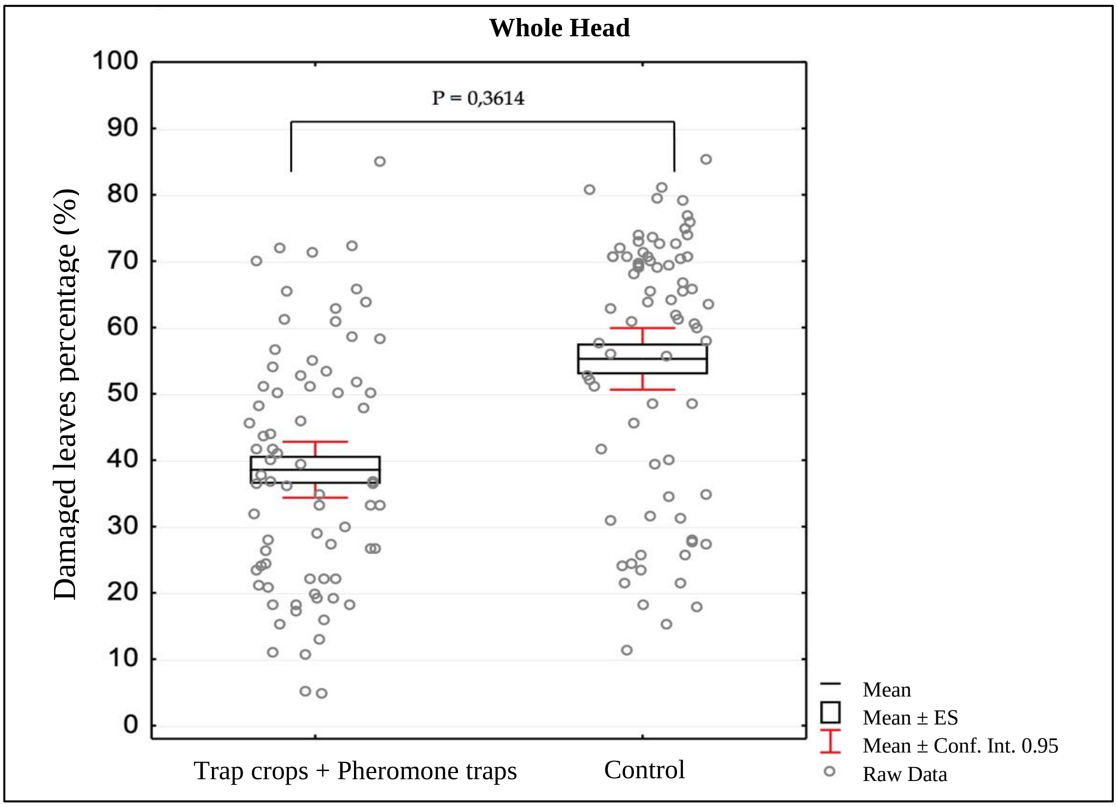

Figure 59 - Percentage of leaves damaged by L. rugulipennis in whole lettuce heads, 2021 growing season.

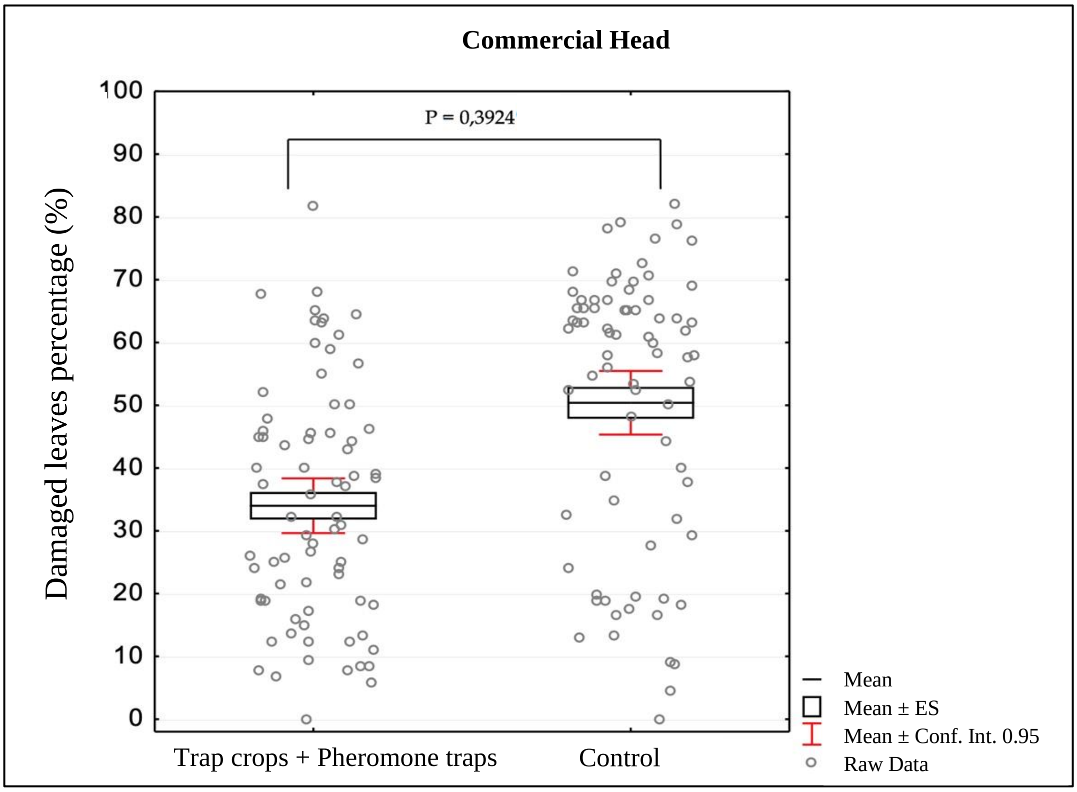

Figure 60 - Percentage of leaves damaged by L. rugulipennis in commercial heads, 2021 growing season.

7. *DISCUSSIONS*

7.1. Trap cropping technique against flea beetles on sugar beets

As regards the experiments aimed at the evaluation of the effectiveness of a trap cropping strategy in containing flea beetle infestations on sugar beets, our trials showed good efficacy in both years of investigation.

Trap crops showed a significant effect in reducing flea beetle damage on sugar beet plants in both years. Pooling the two years, the mean damage index decreased from 37.51% (CI 95% = 33.06–41.97%) on sugar beet grown with a bare soil border to 23.02% (CI 95% = 19.85–26.20%) of the plants with a trap crop border with an overall reduction of 38.62% (Fig. 61).

This effect of reduction of the damage on sugar beet rows produced by the trap crop has been highlighted only from the distance of 6 m from the trap crop border onward. Indeed, at the first sampled rows, which were 2 m from the border, sugar beets presented a damage index that was not statistically different from those placed at the same distance in the control plot. Flea beetles show a characteristic colonization pattern, moving into sugar beet fields from the outer edges, generally crawling on vegetation but also by flying (Burgess, 1977). This pattern explains why, after had fed on the trap crop border, it is likely that flea beetle adults entered in the sugar beet field, feeding first on plants nearer to trap crop, which were located at the distance of 2 m in our study. Thereafter, it is likely that fewer flea beetles will move further into the sugar beet field. For these reasons, flea beetle damage reduction was maximized in sugar beet rows located at higher distances, which were between 6 and 15 m from the trap crops in our study. Although no additional studies on distances over 15 m were conducted, based on flea beetles' colonization pattern and feeding activity, a further decrease in their abundance on sugar beets at increasing distances from the trap crop border can be expected.
Furthermore, in our experiments, the mixture of *S. alba* and *B. juncea* chosen for the trap crop borders proved to be very attractive to flea beetles. Their strong attraction has been highlighted by several unsystematic visual surveys throughout the entire period of the experiments. in which a high density of several flea beetles species as well as a large number of severely damaged leaves were found on these borders.

These findings are of practical importance in order to identify both the mixture of plant species most attractive to flea beetles and the most suitable distance at which trap crop should be sown, namely at least 6 m from sugar beet.

Therefore, on sugar beets, trap crops could be implemented along the fields perimeter in order to create an inward barrier to infestation, acting as a sink for flea beetles, preventing massive movement towards the cash crop.

This study represents, to knowledge, the first attempt to build a pest management strategy for flea beetles on sugar beets based on trap cropping, with the aim of reducing at the same time crop damage and pesticides applications.

However, there are several aspects that must be taken into account beyond the effectiveness of this technique.

First, from a practical standpoint, a crucial aspect to consider, in order to appropriately plan this technique to manage flea beetles in sugar beet, is the trap crop sowing time. Ideally, indeed, trap crops should be sown few weeks earlier than the sowing of sugar beets in the field. In this way, it is possible to match the presence of an attracting development stage of the trap crops with the most susceptible development stages of sugar beets, i.e. from cotyledonary stage to 6 true leaf stage (BBCH16).

An additional very important aspect is the choice of the correct time for trap crop plants termination, which should be scheduled in order to avoid seed production, otherwise they could potentially become weedy plants in the following growing seasons.

For this reasons, once sugar beets have reached a development stage at which flea beetle damage is more tolerated, namely after the stage of 8 true leaves (BBCH18), it is possible to remove trap crops through chopping or mowing. Furthermore, in the specific case of Brassicaceae, trap crops could also serve as green manure, taking advantage of improved soil structure and fertility, of a better weed control, as well as of the biocidal activity against some soil-dwelling insects, such as wireworm larvae, or pest nematodes (dos Santos *et al*, 2021; Furlan *et al*, 2010; Laznik *et al*, 2014).

On the other hand, from an economic standpoint, the implementation of the trap cropping technique obviously implies having to sacrifice parts of the field, that could otherwise be cultivated with a more economically valuable crop. In the specific case of perimeter trap cropping applied on sugar beet cultivation, however, the sacrifice of the outermost parts of fields would not have a significant economic impact, as the sowing of a trap crop border of a 2 m width on the field perimeter would just lead to the loss of the 4 outermost rows of sugar beets, which generally represent the least productive in the whole field (Sparkes *et al*, 1998).

Moreover, from the economic perspective, there are additional logistical and agronomical issues that are caused by the introduction, into the cropping system, of another vegetable species beside cash crop, especially on large fields. Indeed, besides the costs of an additional sowing and mulching, on the trap crop it may be necessary to intervene with emergency irrigation/s or fertilization, with timing that can be different compared to those of the main crop.

In contrast with the aforementioned disadvantages, the implementation of trap cropping on sugar beet, may allow growers to reach a potentially positive cost-benefit ratio, due to the advantages that this technique is capable to offer (and that will be deepened in Chapter 8), such as the decreased pesticide use, the improved soil and environmental quality and the enhancement of the conservation of natural enemies.

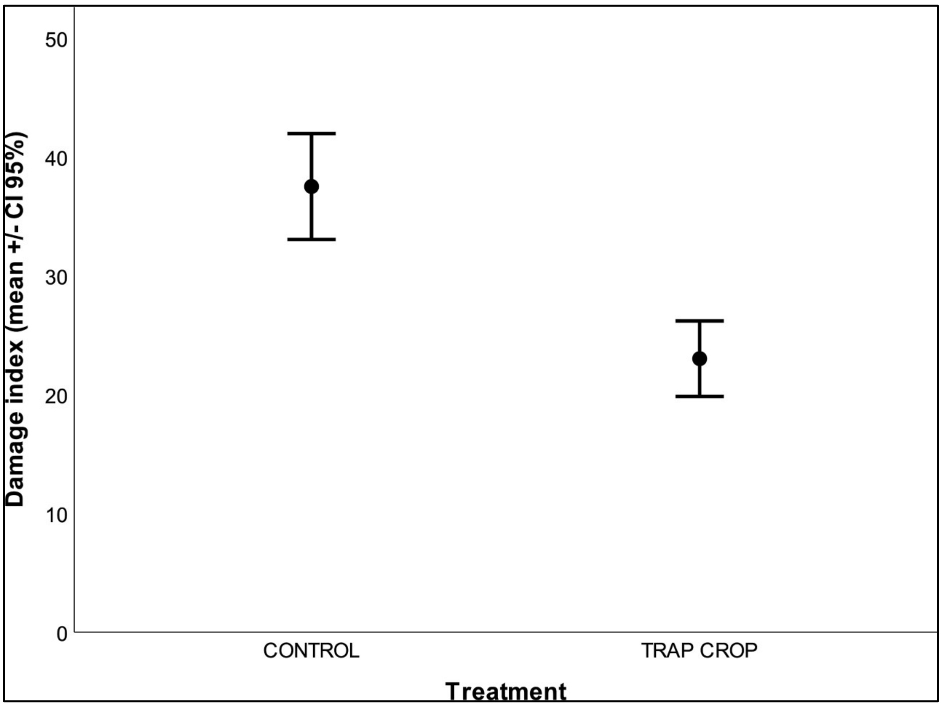

Figure 61 - Damage index due to flea beetles in sugar beet plants as a function of field edge management. The GLMM detected significant differences (P < 0.05) between trap crops and control (bare soil).

7.2. *Lygus rugulipennis* on lettuce

As regards the experiments, aimed at both the evaluation of the most attractive trap configuration towards *L. rugulipennis* on lettuce and at the efficacy evaluation of the agroecological strategy based on the use of semiochemically baited traps implemented into attractive alfa-alfa trap-crop borders, our tests showed interesting results in both years of investigation.

In particular, regarding the trial on pheromones effectiveness and traps placement, the study first highlighted that traps baited with the specific pheromone mixture for *L. rugulipennis* (baited) captured significantly more insects than unbaited (empty) ones.
In baited traps, most of *L. rugulipennis* specimens caught were males, as to be expected due to the sex pheromone used. Still, a large number of females specimens were caught both in baited and in empty traps. This may probably be caused either by the attraction exercised by the male specimens already captured and present inside the trap body or by the colour or shape of the trap, which could somehow attract specimens of *L. rugulipennis* inside it. Indeed, in empty traps, those without the presence of the sex pheromone, both male and female specimens were found captured inside, suggesting a potential attractive power inherent in the used traps, probably due to the combination of their colour, shape, and size.
Another outcome of practical importance of this study is that relating to the best height at which to place the traps. Indeed, our experiments highlighted that traps placed at ground level (low traps) captured significantly higher numbers of *L. rugulipennis* specimens compared to traps placed at the heigh of 70 cm (high traps), indicating that the pest prefers to fly at low heights, nearby the lettuce plants it feeds on. At the same time, traps placed at ground level (low traps) showed lower levels of captures of Coccinellidae beetles, compared to traps placed at a height of 70 cm (high traps), indicating that low traps are the best configuration also from an environmental point of view, minimizing the collateral damage related to the capture of beneficial insects.

Regarding the semiochemical-assisted trap cropping strategy trial, our two-year experiments confirmed the effective attractive power of the alfa-alfa strips towards *L. rugulipennis*, as reported in several studies (Swezey *et al*., 2007; Pansa and Tavella, 2009; Godfrey and Leigh, 1994; Accinelli *et al*., 2005). In the agroecological strategy implemented in our studies, the attraction towards the pest has been enhanced by the placement of traps baited with specific sex pheromone. This strategy significantly reduced *L. rugulipennis* damage on commercial lettuce heads in the first year of studies, from 34.0 % (± 4.9 ES) on lettuce heads of the control plot to 19.8 % (± 1.5

ES) on lettuce heads of the semiochemical-assisted trap cropping plot, with an overall damage reduction of 41.8 %. Conversely, in the second study year no significant differences in the percentage of damage on both the whole and commercial lettuce heads between the agroecological strategy and control plot were highlighted; still a damage reduction from around 50-55 % on lettuce heads of the control plot to around 34-38 % on lettuce heads of the semiochemical-assisted trap cropping plot was reported, with an overall damage reduction of around 30-32 %.

Beside the efficacy of the agroecological strategy implemented in the study, there are several aspects that must be considered.

Among these, the economic aspect surely represents the most important one: the used trapping system, indeed, is highly cost-effective for the monitoring of the pest, but there are further improvements that can be made in order to employ it for mass trapping. For example, an obvious flaw in the traps tested is the lack of an attractant for female specimens of *L. rugulipennis* (Fountain *et al*., 2017), even though, as mentioned before, a certain number of females still went captured. To overcome this issue, phenylacetaldehyde and/or (E)-cinnamaldehyde could be added to the sex pheromone to increase catches of females (Koczor *et al*., 2012). Moreover, the pieces of net impregnated with insecticide employed as killing method in the trapping system, although they proved to be very effective, need to be replaced on a monthly basis, which would be, in the case of mass trapping, a very time-consuming activity. Anyway, among killing methods (which also include drowning solution or biological control agents), this represents the most efficient and easy-to-use one. In addition, also the lures need maintenance as their longevity is approximately four weeks (Fountain *et al*., 2014), then they need to be replaced with new ones. It would be beneficial to increase the longevity of these lures in order to lower time consumption.

Regarding the alfa-alfa strips, from an economic standpoint, they surely represent a cost for the farmers. Indeed, charges for both the sowing and maintenance (i.e., fertilization, irrigation, mowing, termination, burial) of the strips must be incurred. Furthermore, contrary to what was reported for sugar beets, the implementation of alpha-alpha strips in lettuce fields could have a more considerable economic impact, as the need to sacrifice portions of arable land to sow the attractive borders occurs on a highly economically valuable crop.

Anyway, despite the disadvantages, the implementation of this semiochemical-assisted trap cropping strategy has proved to effectively reduce *L. rugulipennis* damage on lettuce by more than 30% in both study years. Moreover, this damage reduction was observed in a period in which, in northern Italy, the populations of *L. rugulipennis* are generally extremely high (Accinelli *et al*., 2002); for this reason, it is reasonable to suppose that the strategy, if applied in periods in which pest pressure is lower, could allow an even greater reduction of lettuce damage. This effective damage containment, on a crop that has a really low damage threshold (aesthetic damage) could lead to decrease the unmarketable lettuce heads percentage, thus allowing growers to reach a potentially positive cost-benefit ratio.

In addition to this, alfa-alfa strips used in this strategy may represent an ideal habitat for various beneficial insects, also including predators and parasitoids of Miridae, such as *Anaphes fuscipennis* Haliday (Accinelli and Burgio, 2002) and *Peristenus digoneutis* Loan (Tavella *et al*., 2002), which are the most common parasitoids attacking *L. rugulipennis* in Italy. Therefore, releases of these parasitoids could be performed on the alfalfa strips, alternatively to the localised use of insecticides against *L. rugulipennis* which have been attracted there. Indeed, on alfa-alfa strips, released parasitoids could find ideal conditions, such as high hosts density and of food sources, allowing growers to simultaneously decrease pesticides use, to improve both soil and environment quality and to contribute to the conservation of beneficial insects.

8. *CONCLUSION*

In 2020 and 2021 growing seasons two different studies have been conducted against flea beetles and *L. rugulipennis*, key pests of, respectively, sugar beets and lettuce. Both studies were carried out using an agroecological approach mainly based on the strategy of trap cropping.

In particular, the study regarding the use of trap cropping in order to manage flea beetles infestations and damage on sugar beets represents the first study attempt to implement this strategy on this crop. Contrarywise, studies on the use of trap crop strategies aimed at controlling *L. rugulipennis* damage on lettuce have been previously carried out, but, in most of them, trap crops were combined either with insecticides applications (particularly dangerous for beneficial insects) or with the use of expensive vacuuming machines, while in our study trap crops have been integrated with environmentally friendly pheromone traps. This represents the first attempt to implement the use of *L. rugulipennis* pheromones in a semiochemical-assisted trap cropping strategy in Italy.

Both studies have demonstrated how the trap-cropping strategy, if correctly applied in terms of plant species used and timing, can actively contribute to the reduction of damage to crops, demonstrating at the same time to allow an effective reduction in the use of insecticide treatments.

Examples of similar successful trap cropping implementation have been reported by other studies conducted on different insect species:

- One of the best examples is the black mustard, *Brassica nigra* L. (Brassicaceae) grown in organic sweet corn (*Zea mays* L.) to control the southern green stink bug, *Nezara viridula* L. (Hemiptera: Pentatomidae), which lead to a decrease in the percentage of damaged sweet corn cobs from 11-22% of the control fields to a 0-1% (Rea *et al*. 2002).

- Another example is the use of perimeter trap crops of blue hubbard squash, *Cucurbita maxima* Duchesne (Cucurbitaceae) to protect butternut squash, *C. moschata* Duchesne (Cucurbitaceae) from *Acalymma vittatum* Fabricius (Coleoptera: Chrysomelidae), which lead to reduce the need to insecticide sprays in the main crop area up to 94% compared with conventional control methods (Cavanagh *et al*., 2009).

- Several studies have successfully employed the technique of trap cropping in order to protect oilseed rape, *Brassica napus* L. (Brassicaceae) from pollen beetles, *Brassicogethes spp* Audisio and Cline (Coleoptera: Nitidulidae). Several Brassicaceae species were the tested as trap crops; in particular, turnip rape (*Brassica rapa* L.) (Gotlin Čiljak *et al*., 2016; Hokkanen *et al*., 1986; Hokkanen, 1989), yellow mustard (*Brassica juncea* L.) (Kaasik *et al*., 2014b), black mustard (*Brassica nigra* L.) (Veromann *et al*. 2012; Kaasik *et al*., 2014a), *Raphanus sativus* L., and white mustard (*Sinapis alba* L.) (Kaasik *et al*., 2014b) proved to be promising as trap crop species.

In most of these studies, tested trap crops demonstrated the ability to effectively attract and retain the targeted insect pests, also pointing out the potential for targeted selective insecticide applications capable of killing important numbers of insects while limiting, or avoiding, the use of broad-spectrum insecticides on cash crops.

Also with regard to our studies, tested trap crops have shown attractiveness towards targeted pests. Indeed, although no specific studies regarding the abundance of pests within the vegetation of the trap crops have been carried out, high densities of both flea beetles and *L. rugulipennis*, as well as a large number of severely damaged leaves have been observed on trap crops through several unsystematic visual surveys.
In our studies, trap crops have contributed, on the one hand, to increase the incomes of lettuce growers, effectively reducing the percentage of unmarketable lettuce heads, whereas, on the other hand, to effectively contain flea beetles damage on sugar beets below the economic damage thresholds. Indeed, in the study area, for the integrated management of flea beetle on sugar beets three action (spray) thresholds are provided (Regione Emilia-Romagna, 2022):

- any holes on cotyledonary leaves
- two holes/leaf on plants with two leaves
- four holes/leaf on plants with four leaves.

The Integrated Production Regulations of the Emilia-Romagna region, provide that, upon exceeding the above-mentioned thresholds, up to 3 specific insecticide applications can be performed against flea beetles.
In our study, thanks to the implementation of trap crop borders, damage caused by flea beetles on sugar beets has always been contained below the action thresholds. Indeed, the number of

holes/leaf observed in the trap crop plot has never exceeded the action thresholds set for the different development stages of the sugar beets (BBCH), thus leading to a potential avoidance of the use of specific insecticide treatments. Contrarywise, in the control plot, number of holes/leaf much closer to the action thresholds were observed and threshold set for BBCH12 were even exceeded in 2021 growing season. This would have led to the potential need for insecticide application.

As far as the defence of lettuce from *L. rugulipennis* is concerned, the Integrated Production Regulations of the Emilia-Romagna region provides for an intervention threshold which is equal to the mere presence of the insect on the crop. For this reason, the presented semiochemical-assisted trap cropping strategy, based on traps baited with sex pheromones installed inside attractive alfa-alfa trap crop borders, could prove to be extremely useful in taking the pest out of the main crop, thus decreasing its presence there and consequently the need for treatment.

Therefore, those practical results show great potential as a viable option for pest management, both in integrated farming but especially in organic farming, where there are even fewer weapons available to rely on for the control of these dangerous insects.

However effective, in order to correctly implement trap crops within the company's typical crop management, it is necessary to make some considerations, analysing both the potential advantages and disadvantages that this strategy can lead to.

General limitations and challenges:

- First of all, in order to establish a successful trap cropping system in different agronomic situations, a thorough understanding is required of both the behaviour and preferences of the targeted insects, as well as the attraction of natural enemies for the trap crop species. Indeed, since different pests show distinct preferences for different plants, a viable trap cropping system is knowledge-intensive, demanding information either on plant species to use as well as their temporal and spatial attractiveness of potential trap crops to maximize their effectiveness. Moreover, the relative attractiveness of the plant species used as trap crops may be influenced by other factors such as the area of field to cover with trap crops, the planting time of both the main crop and trap crops, the phenology of both pest and crop, the physical and chemical properties of the plant used, the proximity between trap crops and main crop, the proportion between trap crops and cash crops in the field, and the climatic conditions of the growing season. For this reason, an in-depth study of each of these factors is required to search for suitable trap crops to be used to control their damage in agriculture.
- Another disadvantage of the trap cropping strategy is that the plants species chosen as trap crops, although they may prove to be particularly attractive to a pest, may not guarantee their retention during the stages of the cash crops most susceptible to damage. Therefore, in future trap cropping systems, retention rates could be improved in trap crops by adding semiochemicals (as we did in our study on *L. rugulipennis*), which has been suggested to enhance trap cropping at a larger spatial scale (Piñero and Manandhar, 2015).

- Another reason why only a limited number of trap crops cases has been implemented at commercial level lies in the fact that, in many cases, agricultural crops are attacked by a complex of insect pests, while trap crops tend to be relatively species specific, making the strategy less practical if compared with the use of broad-spectrum insecticides, able to control a complex of insect pests. Indeed, since farmers have now easy access to insecticides and since their cost is often lower, compared with the cost for trap cropping implementation, many growers often prefer the approach based on easy-to-use, low-cost, and broad-spectrum insecticides applications over more species-specific approaches.

- Also agronomic and economic aspects must be taken into consideration. Indeed, if compared to typical crop management system, there are additional costs and commitments associated with implementing trap crops that must be incurred. Among these, the different sowing periods can imply the need to carry out the same operation on two separate occasions and, in the case of different sowing rates, also the need to use different agricultural machines. Furthermore, after sowing, trap crops must be correctly maintained in order to exploit their attractiveness. In this regards, additional costs for irrigation and fertilization must be incurred. Once trap crops have outlived their usefulness, i.e. at the end of the period of maximum susceptibility of the cash crop to the targeted pest, they need be destroyed and possibly buried, which are expensive and time-consuming operations.

- In some cases the implementation of the trap cropping strategy may even require cooperation between neighbouring growers. Indeed, there might be situations in which neighbour's cash crop is inadvertently put at risk because the trap crop in which pests are harboured in is placed near the property boundary.

- Finally, since pest management practices need to show consistent results and since the success of a trap cropping system, as previously mentioned, is highly variable, depending on multiple factors, thus increasing the risk of economic loss to the grower, there are also limitations in research funding.

Advantages:

First of all, despite their efficacy, conventional agricultural practices often entail detrimental effects on both the environment and human health (Geiger *et al*., 2010). In particular, in addition to the direct costs, there are various external costs derived from the use of broad-spectrum insecticide, including the monitoring and sanitation for contamination of either soils, water, or food, the intoxication of users and workers, the insurgence of insect pest resistance, as well as the deleterious effects on non-target organisms, including pollinators and beneficial insects, fish, and birds (Adler and Hazzard, 2009). These drawbacks related to the use of conventional cultivation practices imply the need for growers to shift to alternative crop management strategies (McCaffery, 1998), introducing more agroecological approaches to achieve, in particular, pest control. Indeed, the time has come to set up decision-making criteria responding to the increasingly important issues of ecology, environment, and health, not forgetting the socioeconomic issues. In this regards, agroecological crop protection (ACP) (Deguine *et al*., 2017) combines several concepts, such as agronomy, ecology, and integrated pest management, aiming to control pests particularly thanks to conservation biological control (CBC) (Begg *et al*., 2017), achievable through vegetative diversification. Since in most of the cropping systems natural enemies are usually one step behind the pests (Ehler and Miller, 1978), the trap cropping strategy could be exploited as a mean of promising conservation biological control. For example, in our study on sugar beets the mixture of Brassicaceae used, not only made it possible to effectively contain the damage caused by flea beetles but also, since S. alba can act as an insectarium plant for ladybirds and hoverflies, as reported by Gospodarek (2021), was able to contribute to the maintenance of biological control against aphids, other key pests of sugar beets.

- Moreover, trap cropping has the advantage of being a highly adaptable strategy to the specific case studies of both pests and respective cash crops, since it can be managed separately from the latter as regards of sowing and termination times, following the biology of the insect. For example, in our study on sugar beets, the destruction of the trap crop occurred in late spring, only when sugar beets were no longer susceptible to flea beetle attacks, while simultaneously preventing pests that had been harboured into trap crops from develop a new summer generation, which, although less economically dangerous, may lead to cause future infestation in following year. Conversely, on pests which have multiple harmful generations, trap crops can be destroyed just before the developing of the new generation, preventing pest migrations on main crop (Boucher and Durgy, 2004).

- Another advantage of trap cropping strategy is that, in situations in which it has been successfully implemented, it has represented a sustainable and long-term solution to the management of pests that are difficult to control even through the use of conventional methods. In particular, this strategy represents one of the best options for pest management especially in developing countries, where conventional alternatives are either not available or are too expensive. Therefore, trap cropping strategy represents one of the safest solutions to control harmful pests to agricultural crops important for the economy of the country.

- Beside the above-mentioned advantages related to pest management, the implementation of a trap cropping strategy also entails several benefits regarding the improvement of both soil and water quality (Dabney 1996; Dabney *et al*., 2001). These, depending on plant species used as trap crops, can include soil erosion protection, soil enrichment, nutrient leaching reduction, carbon sequestration, weed suppression, losses of nutrients, pesticides, and sediment reduction. For example, the mixture used as trap crop in our study on sugar beets, being composed of Brassicaceae species, once it has been destroyed and buried, releases glucosylates into the soil which, through enzymatic hydrolysis, produce isothiocyanates and nitrile, compounds with a biocidal action against nematodes and insect larvae harmful to sugar beet, such as wireworms (dos Santos *et al*, 2021; Furlan *et al*, 2010; Laznik *et al*, 2014). Furthermore, the abundant supply of organic matter coming from these biocidal plants once buried in soil, brings benefits to both soil structure and fertility. In the case of the study conducted on *L. rugulipennis*, the trap crop consisted of alfa-alfa, which being a plant species belonging to the family of Leguminosae, made it possible to bring high quantities of nutrients, especially nitrogen, to the soil in anticipation of the crop of the following year.

- Finally, as mentioned before, trap crops trap crops certainly give rise to additional costs for growers if compared to those normally incurred by a typical crop management. However, these additional costs turn out to be very often lower, if compared to those deriving from the greater number of phytosanitary treatments normally carried out, the possible costs of soil and water remediation, and the costs, not quantifiable in monetary terms, to be incurred for the damages caused to the environment, biodiversity and beneficial organisms.

Concluding, following the analysis of most of the positive and negative aspects related to the implementation of a trap cropping strategy, it is possible to assess that despite the well-known disadvantages, it may consent growers to decrease the use of pesticides, first reducing their costs, whilst favouring the conservation of natural enemies, and improving both soil and environment' s quality, reaching a potentially positive cost-benefit ratio.

Through our studies, indeed, we demonstrated that trap crops, even if not supported by insecticide sprays, can effectively reduce the damage caused by both flea beetles and *L. rugulipennis* on sugar beets and lettuce, respectively. Therefore, our experiments showed that trap cropping could represent a sustainable strategy to reduce early crop damage caused by flea beetles on sugar beets, lending itself well principally in organic farming, where flea beetle damage is particularly dangerous, due to the few weapons available to contain it.

In the case of lettuce in organic farming, this is even more true, since on lettuce, being a crop intended for direct consumption, unlike sugar beets, it is not possible to carry out insecticide treatments even in the presence of the pest. Therefore, the semiochemical-assisted trap cropping strategy we put in place could represents the only possible weapon to counteract the damages caused by *L. rugulipennis* for organic horticulturists. Furthermore, thanks to the implementation of this strategy, based on alfa-alfa strips, it would also be possible to improve conservation biological control against aphids, other dangerous pests of lettuce crops, for which, similarly to *L. rugulipennis*, there are no great alternatives in organic farming.

These are the main reasons why trap cropping already represents the most common method of pest management used in organic farming (Shelton and Badenes-Perez 2006) and why there is a growing interest from organic growers and those farmers interested in biologically based pest management programs to consider implementing such strategies in their farms.

However, fundings and lack of research may continue to be limiting factors for trap cropping strategies development, but new opportunities are emerging, as the CAP is increasingly pushing towards a greener approach, allocating an increasing majority of payments to measures in favour of the environment, climate and biodiversity. Indeed, in the new CAP 2023-2027 at least 25% of the direct payments budget will be allocated to eco-schemes, providing more incentives for climate-friendly and environmentally friendly agricultural practices and approaches (such as organic farming, agroecology, carbon sequestration in agricultural soils, etc.) and at least 35% of the funds will be allocated to measures supporting climate, biodiversity, the environment and animal welfare. In this context, the trap cropping strategy could potentially represent a measure eligible for funding, thus representing a gain for the farmer also from an economic standpoint and not only from the pest management point of view. Moreover, this greater potential for applicability of trap cropping could lead to more academicians, development workers, and scientists to better study the strategy, increasing knowledge and perhaps even extending it to new pests that are difficult to control by other means.

Nevertheless, the technique requires additional studies to be focused on evaluating the influence of the timing of both sowing and removal of the trap crop plants, in order to improve the application of this strategy in each receiving environment. Further study is also needed on the effective application of trap crops, including cropping pattern (e.g., perimeter, sequential, multiple and push-pull planting schemes), the total percentages compared to the cash crop, as well as maintenance details.

Finally, to develop the trap cropping strategy to its full potential a multifaceted approach involving research and communication is required.

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