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### The value of flower strips for the biological control of aphid vectors of sugar beet yellows

Ludovic LAGNEAU<sup>1</sup>, Cécile LE LANN<sup>1</sup>, Kévin TOUGERON<sup>3,4</sup>, Elsa CANARD<sup>2</sup>, Ronan MARREC<sup>3</sup>, Manuel PLANTEGENEST<sup>2</sup>, Anne LE RALEC<sup>2</sup>, Joan VAN BAAREN<sup>1</sup>, Yann TRICAULT<sup>2</sup>

- <sup>1.</sup> University of Rennes, UMR-CNRS 6553 ECOBIO, France
- 2. Institut Agro Rennes Angers, UMR INRAE University of Rennes 1 IGEPP 1349, France
- 3. University of Picardie Jules Verne, UMR-CNRS 7058 EDYSAN, France
- <sup>4</sup> University of Mons, EICG, Biosciences Research Institute, Belgium

Corresponding author: joan.van-baaren@univ-rennes1.fr

**Abstract**: The ban of neonicotinoids makes it compulsory to implement alternative strategies for the management of aphids that carry sugar beet yellows. The installation of flower strips near crops could contribute to a better biological regulation of aphids by favouring their natural enemies through the provision of food resources (nectar, pollen, alternative prey etc.) and habitat. The analysis of 58 studies devoted to flower strips in field crops confirms their capacity to increase the abundance of parasitoids or predators and to reduce pest infestations. A second corpus of 32 publications provides a first, yet incomplete, list of natural enemies of the main aphid vectors of beet yellows. Among the factors influencing the effectiveness of a flower strip on biological control, the specific composition and the floral traits of the species of the mixture are decisive. The influence of the landscape and climatic contexts has been little discussed so far. The results collected from the literature seem to indicate that the composition of the flower strips to be recommended in sugar beet crops could differ between temperate or oceanic climates and continental climates. Fieldwork is still needed to assess the relevance of the flower strip approach to the management of yellows.

**Keywords**: agroecological infrastructure, virus, parasitoid, predator, landscape, climate

#### Introduction

A quarter of the world's sugar comes from sugar beet, *Beta vulgaris* (Biancardi *et al.*, 2010; Heno *et al.*, 2018). France is the second largest producer, with some 400,000 hectares devoted to the crop. Viral yellows are one of the main phytosanitary problems affecting sugar beet. Four virus species, transmitted to the plant by aphids (BYV, BYMV, BChV, BtMV), are responsible for these diseases (Hossain *et al.*, 2021). The main vectors of these viruses are the green peach aphid, *Myzus persicae*, and the black bean aphid, *Aphis fabae* (Hossain *et al.*, 2021). Other more sporadic aphid species, such as *Aulacorthum solani*, *Macrosiphum euphorbiae*, *Myzus ascalonicus*, *Myzus ornatus* and *Rhopalosiphoninus staphyleae* (the scarce beet aphid), can also transmit viral yellows.

Viral infection damages the photosynthetic mechanism, leading to a reduction in net photosynthesis and to leaf yellowing symptoms. It is also associated with reduced growth of lateral roots and a significant weight loss of the entire plant, including the taproot. All these processes reduce the amount of sugar extracted from infected plants (Clover *et al.*, 1999). Yield losses vary depending on the virus species involved (Hossain *et al.*, 2021). Susceptibility to the diseases diminishes as the plant grows, through a mechanism of resistance to aphids at maturity (Schop *et al.*, 2022). However, the epidemiology of yellows in the field remains poorly understood. In France, the severity of infestations and the prevalence of the various viruses vary widely from one department (region) to another (ITB, 2021). In the absence of effective plant protection, up to 100% of beet can be symptomatic. The average yield of beet affected by yellows decreased by 24% in 2020 and 20.5% in 2021, compared to asymptomatic beet grown in the



same fields (ITB trial on 26 fields - ARTB, 2022). In 2020, in a context of ineffective management of yellows and high sanitary pressure (early and abundant flights of aphid vectors), national beet yields fell by 29% (ANSES report, 2021).

Since the 1990s, the management of beet yellows in conventional systems has been based entirely on vector control using neonicotinoid insecticides as seed treatments (Hauer *et al.*, 2017; Hossain *et al.*, 2021). This strategy provided effective aphid control during the crop's sensitive period (from sprouting to row closure). At the end of the 2000s, many voices were raised to denounce the negative impact of the use of neonicotinoids on non-target organisms such as pollinators (Goulson, 2013; Hauer *et al.*, 2017). In France, Law n°2016-1087 of August 8th, 2016 "for the reconquest of biodiversity, nature and landscapes", recognised this environmental risk and led to a ban of neonicotinoids from September 1st, 2018. However, a derogation for sugar beet crops meant that they could still be used in 2021 and 2022.

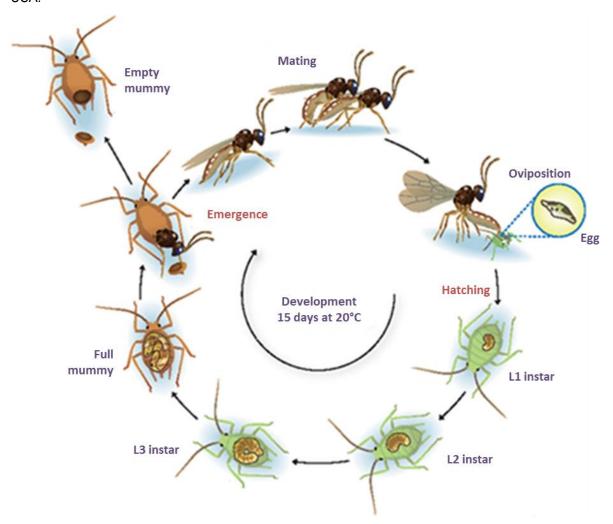
To compensate for the withdrawal of neonicotinoids from beet crops, various alternative methods can be proposed and combined (Jactel *et al.*, 2019; ANSES 2021; Francis *et al.*, 2022; Verheggen *et al.*, 2022). They focus mainly on the control of aphid vectors and include in particular: (i) the use of aphicides, (ii) the use of Volatile Organic Compounds (VOCs) acting by attraction or repulsion of aphids, or by attraction of their natural enemies, (iii) the use of physical methods (visual confusion, passive traps, physical barriers....), (iv) the deployment of sugar beet varieties resistant to viral yellows and/or to aphid vectors, and (v) biological control, which aims to regulate aphid vector populations by applying entomopathogenic micro-organisms (fungi, viruses or bacteria) or macro-organisms (parasitoids and/or predators), or by encouraging their natural enemies through agro-ecological approaches (creation or conservation of seminatural habitats, crop associations).

The French National Research and Innovation Plan (PNRI) "Towards operational solutions against sugar beet yellows", launched in 2021 by the French government, aims to evaluate all the alternatives to neonicotinoids with a view to their operational use by the end of 2023. The IAE-Betterave project (AEI for Agro-Ecological Infrastructure), funded by the PNRI, seeks to assess the value of flower strips for the biological regulation of aphids. In this paper, we present the general mechanisms involved and analyse the factors that vary their effectiveness, linked to the characteristics of the flower strips and the landscape and climatic contexts in which they are planted. Finally, we outline possible ways of adapting this agroecological approach to the management of viruses in sugar beet. As very few references exist for this crop, we based our work on a semi-quantitative analysis of the scientific literature on the effects of flower strips on biological control. This work complements recent reviews devoted to AEIs, which are either qualitative and general (see for example Gurr et al., 2017) or quantitative but based on a relatively small number of works (18 publications for Albrecht et al., 2020). We studied how flower strips or their characteristics influenced variables measuring pest regulation by counting positive, negative or neutral effects, in the agronomic sense. For example, an increase in the number of natural enemies in the crop close to the flower strip compared with a control situation was considered a positive effect. A corpus of 45 publications published between 1993 and 2021 was compiled using a keyword search on Google Scholar and Web of Science, supplemented by the documentary resources of various partner organisations. The method is detailed in Appendix 1, which also provides a list of the publications obtained. The publications finally selected for analysis were those presenting usable quantitative data (biological control measurements), obtained in field crops, with a comparison of flower strips and control strips (bare soil or spontaneous vegetation or cultivated vegetation identical to the crop in the field) installed at the edge of the field and mainly in Europe. The majority of studies (54%) focused on cereal crops, 17% on oilseed rape and 9% on potatoes. Beetroot, soyabean, peas, cabbage and maize were represented by 2 to 4 studies each. Among the pests studied, aphids featured in 17 studies. A variety of natural enemies were studied, but ground beetles (n = 34 studies), ladybirds (25), spiders (23) and parasitoids (18) were studied more systematically.



#### **Conservation Biological Control and resources for natural enemies**

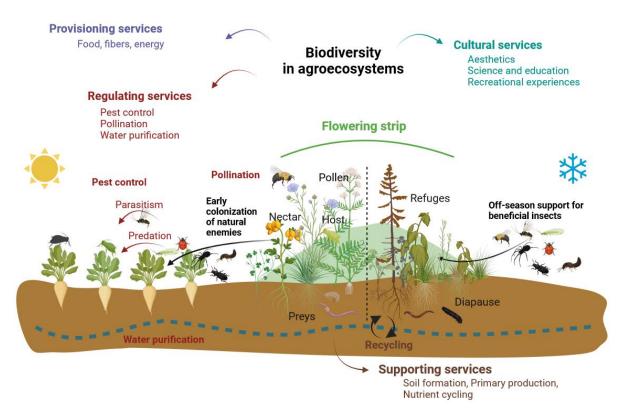
In agroecosystems, insect pest populations are regulated by a range of natural enemies belonging to two distinct functional groups: predators (notably ladybirds, carabid beetles, hoverflies and spiders) and parasitoids (Le Ralec *et al.* 2019). Parasitoid females actively forage for hosts to lay their eggs, and the larvae develop by consuming the host (Figure 1). Aphids are exploited by numerous species of solitary parasitoid Hymenoptera (a single larva per aphid), whose abundance and activity vary according to a range of manageable properties of agro-ecosystems, such as the agronomic practices applied, crop diversity, landscape structure and the characteristics of semi-natural habitats. In a context of widespread insecticide use, the potentially considerable impact of natural enemies on pest populations can go unnoticed. Landis *et al.* (2008), for example, estimated that the natural regulation of the soy aphid, *Aphis glycines*, saved around 131 million dollars a year on just 28% of the area under soybean cultivation in the USA.



**Figure 1:** Development cycle of an aphid parasitic wasp. In many species, the adults feed on nectar (floral or extrafloral). Honeydew produced by Hemiptera is another potentially important source of sugars. Source: Encyclop'Aphid (translated from the source)

Conservation Biological Control (Figure 2) involves promoting the pest regulation service provided by their naturally occurring natural enemies (Holland *et al.*, 2016).





**Figure 2:** Contributions of a flower strip to ecosystem services. Focus on the crop pest regulation service used in Conservation Biological Control (creation: BioRender.com)

This strategy is based on the one hand on reducing mortality factors (e.g. by insecticide treatment) and on the other hand on improving the resources available to predators and parasitoids in and around crops, up to the scale of the agricultural landscape (from a few km² to a few tens of km²) (Barbosa, 1998; Landis et al., 2000; Holland et al., 2016). The different types of resources are directly or indirectly provided by cultivated (e.g. pure crop, associations, agro-forestry) or non-cultivated (e.g. weeds, hedgerows) plants and include:

- (i) food resources produced by flowering and/or nectar-producing plants and consumed by adults. These are nectar, an important source of sugars (sucrose, glucose and fructose) and water (Heil, 2011), and pollen, a source of amino acids and proteins. Honeydew from aphids, whether or not they are fed by the crop, is another source of sugars that is often abundant and easily accessible. Frequently exploited by parasitoids and hoverflies, these resources can increase the longevity, mobility and fecundity of females, leading to an increase in the rates of predation or parasitism suffered by pest populations (Gurr et al., 2017; Damien et al., 2020; Thomine, 2019). Improved performance has been documented, for example, in Brassica aphid parasitoids accessing extrafloral nectar in the presence of their hosts (Jamont et al., 2013),
- (ii) alternative hosts/prey hosted by "reservoir plants" (or relay plants). These resources facilitate the early establishment of natural enemies in the crop or its immediate vicinity, enabling them to maintain or even multiply when the target pest is scarce (Landis *et al.*, 2000; Gurr *et al.*, 2017). In some aphid parasitoids, however, a marked level of specialisation limits the interest of alternative hosts in pest regulation (Derocles *et al.*, 2014),
- (iii) habitats with little disturbance and/or favourable microclimatic conditions (temperature and humidity), offering protection against heatwaves, for example, or shelter from agricultural disturbances (treatments, tillage, etc.) (Landis *et al.*, 2000; Gurr *et al.*, 2017; Damien, 2018;



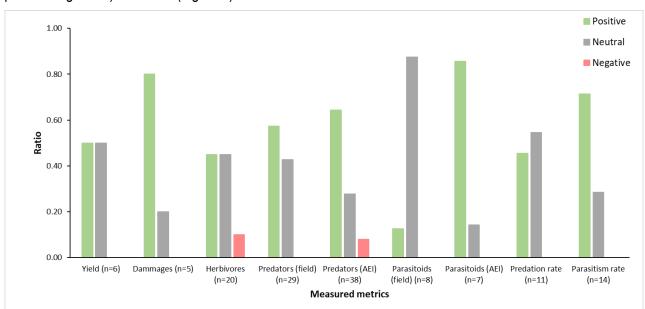
Thomine, 2019). This refuge function is important in temperate areas where many arthropods involved in biological control overwinter outside crops (Geiger *et al.*, 2009).

Conservation biological control can be enhanced by the preservation and establishment of AEIs that diversify plant communities and increase the quality and quantity of resources available within the agricultural landscape (Landis *et al.*, 2000). These semi-natural habitats include punctual elements such as isolated trees and ponds, linear elements such as flower strips, grass and weed strips or hedges including riparian zones, and landscape features such as woods and copses, wetlands, extensive grasslands, flower fallows and even intercrop cover (Damien *et al.*, 2017; Hatt *et al.*, 2018; Thomine, 2019). Together, these AEIs form a network of little-disturbed habitats, often managed by farmers, whose good functional state guarantees the provision of ecosystem services. Conservation biocontrol, based on improving the resources provided by AEIs, is likely to promote other types of service, such as pollination or soil and water conservation (Figure 2).

#### Flower strips, AEIs for pest control

Flower strips are AEIs set up by farmers with the main aim of improving biological control of pests and/or crop pollination. As part of an agro-ecological approach, they should help to limit the use of insecticides. The service plants they contain are attractive to natural enemies, offering them abundant nutritional resources (nectar, pollen, honeydew from Hemiptera). They also host prey and alternative hosts and act as shelters (Twardowski *et al.*, 2005; Walton & Isaacs, 2011; Damien, 2018; Albrecht *et al.*, 2020; Hatt *et al.*, 2020).

We identified 45 publications including a total of 58 studies on the effect of flower strips on pest regulation in field crops, a third of which concerned aphid crop pests. These effects were generally positive (improved pest management) or neutral (Figure 3).



**Figure 3**: Proportion of studies showing positive (in the agronomic sense, i.e. beneficial to the crop, in green), neutral (in grey) or negative (in red) effects of flower strips on the variables measured: crop yield, pest damage, abundance of herbivores, predators and parasitoids in fields or flower strips, predation rate and parasitism rate. n indicates the number of studies in which the variable was present, out of the 58 studies taken from the 45 publications analysed.

The abundance of natural enemies in flower strips, the variable most frequently measured, was very often higher than in the controls. This effect is rarely found for the abundance of parasitoids in the crop but,



interestingly, the rate of parasitism of crop pests is generally higher. This suggests that adult parasitoids use flower strips to feed (and/or multiply on alternative hosts). They are therefore more often captured or observed there, but also explore the field to exploit their hosts (lay their eggs). This suggests that the two types of habitat and resources complement each other, through regular back and forth movements of the female parasitoids. The flower strips also tend to increase the abundance of predators, including in the field, without any systematic improvement in the rate of pest predation in the field being measured. However, predation is more difficult to quantify than parasitism, which in the case of aphids is evidenced by the transformation of the host into a mummy when it dies (Figure 1). The increase in predator and parasitoid populations is accompanied by a reduction in pest abundance in around half the cases. Finally, few studies looked at damage and yields, but all found at best an agronomic benefit and at worst no effect from flower strips. While we cannot rule out a publication bias in favour of studies showing a significant effect of flower strips, these results corroborate those obtained by Albrecht et al (2020) based on a quantitative summary of 18 studies carried out in various agricultural contexts in Europe, the United States and New Zealand. These authors estimated that enriching the local community of natural enemies with flower strips increased the biological control measured in neighbouring crops by an average of 16% (with no change in yield, however, either upwards or downwards). Interestingly, the effect of hedgerows was comparable in trend but not significant.

Although in a very small minority, two publications report problematic but unresolved situations in which the presence of a flower strip is associated with an increase in pest abundance despite a higher abundance or diversity of predators and a higher rate of parasitism (Denys and Tscharntke, 2002; Török et al., 2021). In 4 other publications, a reduction in the abundance/diversity of predators in the AEI was also shown, but without any effect on their abundance in the field and without the consequences on pest abundance being quantified (Figure 3). These results point to expected limitations (Tscharntke et al., 2016; Damien, 2018) when the design of the flower strip (e.g. choice of flower species) is poorly adapted to the phytosanitary situation or the local context (climate and landscape, see below). Intraguild predation and higher trophic interactions could also limit the positive role of flower strips. For example, hyperparasitoids, which exploit primary parasitoids, may also benefit from a nectar source close to cultivated plants (Jeavons et al., 2021).

Another area of analysis concerns the spatial extent of the effects produced by the flower strip. The dispersal capacity of parasitoids and certain predators often does not exceed a few dozen metres. In addition, the high attractiveness of the flowering mix may limit the propensity of natural enemies to move away to search in the adjacent crop, particularly in the case of female parasitoids which have to alternate between searching for nectar and searching for hosts. Albrecht *et al* (2020) showed that, even when improved by the presence of a flower strip, the level of biological control decreased exponentially with distance from the edge of the field. This phenomenon could explain some of the neutral results observed in our analysis (Figure 3). However, pests frequently colonise the edges of plots before reaching the centre of the field (Tougeron *et al.*, 2022). Flower strips, despite their limited range of action, could therefore be effective in limiting the spread of pests into the crop from its borders.

#### Variation factors in the effect of flower strips on pest control

Several characteristics of flower strips influence their effectiveness and should be considered before they are planted (Albrecht *et al.*, 2020; Tschumi *et al.*, 2016a; Tschumi *et al.*, 2015; Wäckers & Van Rijn, 2012). These include floral traits and nectar quality, linked in particular to the specific composition of the mixture, the longevity of the plants, the size of the strip, its age and the period at which it is planted. These characteristics could be adapted to suit the local climatic conditions.

Hymenopteran parasitoids and hoverflies have short mouthparts that allow them to exploit only flowers with short corollas, which are generally small (e.g. buckwheat flowers). Consequently, floral morphology is a criterion to be taken into account when composing the flowering mix (Baker & Baker, 1983; Petanidou,



2005). Some plant species, such as blueberry or vetch, are also capable of producing directly accessible extra-floral nectar (Damien, 2018). In addition, the volume of nectar produced per flower and per plant, as well as its composition and sugar concentration, vary greatly between species (Heil, 2011). Some sugars have a greater effect on fecundity and others on the longevity of parasitoids (Tompkins *et al.*, 2010; Damien *et al.*, 2020). These differential effects on life history traits (Wäckers, 2001) could be taken into account when composing a flowering mixture adapted to the natural enemies to be favoured. However, although nectar quality partly determines the insect community visiting a flower species (Baker & Baker 1983; Petanidou, 2005), other characteristics may explain its attractiveness (Russel, 2015). For example, yellow mustard is more attractive to parasitoids than white buckwheat, whose nectar quality is nevertheless superior (Damien *et al.*, 2019). Honeydew produced by hemipterans in the flower strip can also supplement nectar resources.

The studies analysed (see also Wäckers & van Rijn, 2012) provide a wealth of information on the specific composition of flower strips. They enable us to identify a number of species that are frequently associated with a positive impact on pest control: *Daucus carota* (Apiaceae), *Achillea millefolium* (Asteraceae), *Centaurea cyanus* (Asteraceae), *Chrysanthemum leucanthemum* (Asteraceae), *Lotus corniculatus* (Fabaceae), *Trifolium pratense* (Fabaceae), *Papaver rhoeas* (Lamiaceae) and *Fagopyrum esculentum* (Polygonaceae) (Table 1). However, this indicative list needs to be adapted to the local context, the target pests and their preferred natural enemies.

**Table 1:** Plant species most often associated (more than 10 times) with a positive effect of flower strips on the different variables linked to the biological control of pests. Calculations based on a compilation of data from 58 studies drawn from 45 publications.

Family	Species	Yield	Damage	Herbivores	Predators	Parasitoids
Apiaceae	Anethum graveolens		2	3	6	
	Coriandrum sativum		2	4	5	
	Daucus carota				10	4
Asteraceae	Achillea millefolium	2		4	12	5
	Anthemis arvensis		2	3	5	
	Centaurea cyanus		3	5	14	4
	Centaurea jacea		2	3	6	2
	Chrysanthemum leucanthemum	2		4	10	4
Cichorioidae	Cichorium intybus	2		2	6	
Fabaceae	Lotus corniculatus	2		3	8	2
	Trifolium pratense			2	7	3
Papaveracea	Papaver rhoeas		3	5	10	2
Polygonaceae	Fagopyrum esculentum	2	3	5	13	3

Of the 51 studies (88%) which mentioned the width of the flower-bedded strip, 33 concerned rather narrow strips, between 1.5 m and 5 m wide, and 18 concerned wider strips, up to 25 m wide in some studies. Irrespective of the variable selected (abundance of pests, natural enemies, parasitism or predation rates), no effect of the flower strip belonging to one or other of these two classes could be demonstrated.

The effect of the age of the flower strips can be examined on the basis of the 47 studies (81%) specifying it. Flower strips more than one year old (n = 20) are more often associated with an improvement in crop



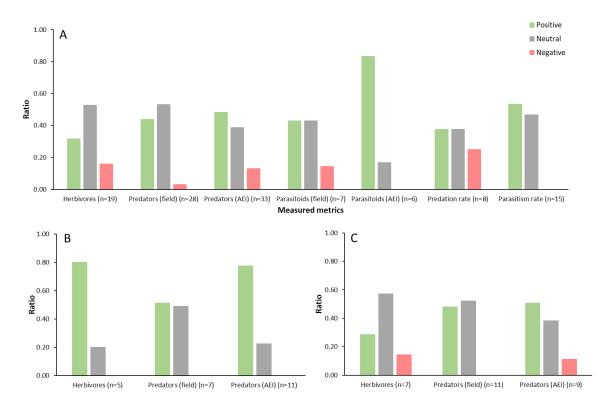
health than those less than one year old (n = 27). This result contradicts the lack of effect of age observed by Albrecht *et al.* (2020) on a more restricted corpus of studies. No particular effect of the types of species making up the flower-bed, annual species (n = 20), perennial species (n = 8) or both types (n = 22), emerged from the analysis of the 50 studies mentioning this factor.

In most of the studies analysed, the flower strips were planted to flower in spring. The few experiments carried out in regions with mild winters show that it is possible to plant flower strips composed of annuals in autumn, for winter flowering (Damien *et al.*, 2017). The presence of prey and alternative hosts, as well as nectar, in these flower strips could contribute to the early development of natural enemy populations (Damien, 2018; Tougeron *et al.*, 2018).

The small number of studies available has not made it possible to assess the effect of the methods used to install the flower strip (sowing date, position at the edge of the field or in the field, etc.). Generally speaking, the technical feasibility and acceptability to farmers must be taken into account, encouraging co-design work. The plant species chosen must be inexpensive to buy seed for, undemanding, preferably not host to pests (or play a marginal role in infesting the crop) and not present a risk of dirtying the crop. In the case of perennial strips, the mix of species must be sufficiently generic to cover the different croppest complexes that follow one another in the rotation, and not be a reservoir of pests.

Because it acts jointly on the phenology of plants (e.g. flowering period) and insects (e.g. diapause period), climate also appears to be an important factor to take into account. A time lag between the availability of the resources offered by AEIs and the outbreak of pests in the field could limit the suppressive effect of the regulatory service. However, the climatic context is largely neglected in studies of flower strips and, more generally, of AEIs. The experiments are usually conducted in a given context. In order to analyse the effect of climate on the basis of a satisfactory number of combinations between variables measuring pest regulation and type of climate, we extended our literature search to studies looking at the effect of grass strips or hedges. Of the 65 publications obtained (link to list in Appendix 1). we selected 60 (17 of which did not deal with flower strips) corresponding to studies carried out under conditions favourable to beet growing. Their climatic context was characterised as oceanic (n = 53)studies), continental (18) or transitional temperate (18). A third of these studies concerned aphid pests. Few variables (n = 3) were measured in temperate or continental climates and none related to parasitoids. More variables are available for oceanic climates (7). Whatever the climatic context, the frequency of positive effects of AEIs far outweighs that of negative effects (Figure 4). In an oceanic climate, the effects on parasitoids are particularly favourable. This result could be attributed to the loss of winter diapause in mild conditions, which allows early colonisation of crops (Tougeron et al., 2018, 2022), and/or to better development of AEIs.





**Figure 4:** Proportion of studies revealing positive (in the agronomic sense, i.e. beneficial to the crop, in green), neutral (in grey) or negative (in red) effects of AEIs (flower strips, grass strips, hedges) on the different variables linked to the biological control of pests. (A): studies conducted under oceanic climate (n = 53); (B) temperate climate (n = 18); (C) continental climate (n = 18).

#### Developing the flower strips approach in a beet context

To design flowering mixtures that help regulate  $Myzus\ persicae$  ("green aphid") and  $Aphis\ fabae$  ("black aphid"), the main causes of yellows epidemics in sugar beet, it is useful to identify their main natural enemies. In Europe and for all crops combined, 31 species of natural enemies are mentioned in the literature for M. persicae and 21 species for A. fabae (Table 2; see Appendix 1 for the literature search method). More parasitoid species attack green aphids (n = 20) than black aphids (n = 7). Two frequently mentioned species,  $Aphidius\ colemani\ and\ Lysiphlebus\ testaceipes$ , are common to both aphid hosts. Predators of the black aphid include numerous species or genera of ladybirds (n = 9; the most frequently mentioned is  $Hippodamia\ variegata$ ), three species of predatory bugs and one species of lacewing. Only two species of ladybird were recorded as predators of the green aphid, along with two species of bugs and cantharids and five species of ground beetle. The predator assemblages are therefore relatively distinct, although three generalist species are common to both aphids (seven-spotted ladybird and bugs of the Anthocoris genus).

**Table 2:** Inventory of natural enemies (NE) of the 2 main aphid vectors of yellows viruses in sugar beet, <u>on all crops</u>. Occurrences were counted in 12 publications for *Myzus persicae* and 22 articles for *Aphis fabae*. In bold, the ENs shared by the 2 aphids.

Type of NE	NE species	Occurrences of NE		
		Aphis fabae	Myzus persicae	
Parasitoid	Aphidius colemani	3	h6	



_	Aphidius matricariae	2	1
	Aphidius avenae		1
	Aphidius ervi		1
	Aphidius nigripes		1
	Aphidius rhopalosiphi		1
	Aphidius transcaspicus		1
	Aphidius uzbekistanicus		1
	Aphelinus asychis		1
	Aphelinus semiflavus		1
	Binodoxys angelicae	1	
	Binodoxys similis		1
	Charipinae sp.	1	
	Dandrocerus sp.	1	
	Diaeretiella rapae		3
	Ephedrus persicae		1
	Ephedrus plagiator		1
	Lysiphlebus fabarum	4	
	Lysiphlebus testaceipes	2	3
	Praon abjectum		1
	Praon myzophagum		1
	Western Praon		1
	Toxares deltiger		1
	Toxares shigai		1
Predator	Anthocoris nemoralis	1	1
	Anthocoris nemorum	1	1
	Cantharis lateralis		1
	Cantharis rufa		1
	Cheilomenes propinqua	2	
	Chilocorus calvus	1	
	Chrysoperla carnea	2	
	Coccinella septempunctata	2	1
	Coccinella undecimpunctata		1
	Exochomus spp.	1	
	Harmonia axyridis	1	
	Henosepilachna spp.	1	
	Hippodamia convergens	1	
	Hippodamia variegata	5	



Orius albidipennis	2	
Propylaea quatuordecimpunctata	1	
Bembidion lampros		1
Harpalus rufipes		1
Patrobus atrorufus		1
Pterostichus cupreus		1
Trechus quadristriatus		1

It is clear that these species lists are incomplete. No species of hoverfly or spider is mentioned, for example. To identify the natural enemies active in beet crops, trapping campaigns are being carried out at various sites as part of the IAE-Betterave project. These should provide new information on the overall level of regulation exerted at the expense of aphid vectors, as well as on the rate of parasitism associated with each of the parasitoid species encountered. Observations made in 2022 have already revealed parasitism of the two aphids by the parasitoids *Aphidius matricariae* and *Diaeretiella rapae*, although this second species was not associated with *A. fabae* in our bibliographic corpus. Taken together, these data will enable us to refine the profile of flowering plants to be mixed in strips according to the resources to be provided. The generalist nature of aphid vectors of viruses will be a major constraint on the choice of flower species to be installed.

The case of the parasitoid *A. matricariae is a* good example of how knowledge of biotic interactions could serve the flower strip approach. *Aphidius matricariae* is part of a complex of biotypes of uncertain status (Derocles *et al.* 2016) capable of exploiting alternative hosts on Poaceae in the winter period and parasitising *M. persicae* on winter oilseed rape and intercrop Brassicaceae. Practices allowing the installation of this complex before beet emergence could contribute to the management of aphid vectors of yellows but also of other aphids in neighbouring crops. However, care should be taken to ensure that host specialisation does not prevent cross-use of alternative host aphids in the strip and target aphids in crops. Buckwheat, mustard, blueberry and faba bean are floral species that can favour this parasitoid (Damien, 2018). However, mustard is a host plant for green aphids on sugar beet.

An original aspect of the IAE-Betterave project is to assess the influence of the landscape and climatic context on the effect of a flower strip on aphid control. The sugar beet production area is vast and covers contrasting territories. The landscapes are sometimes marked by a syndrome of agricultural intensification (poverty of semi-natural habitats, short rotations, heavy use of inputs) generally associated with a loss of cultivated and spontaneous plant biodiversity (Jeavons, 2020), which can affect the natural enemies of aphids, which move around the agricultural landscape mosaic in search of resources. However, the impact of the landscape varies according to the distribution of resources and the dispersal capacity of each organism (Fahrig *et al.*, 2011). The installation of flower strips in the beet-growing zone will make it possible to test the 'intermediate complexity landscape' hypothesis (Tscharntke *et al.*, 2012; Martin *et al.*, 2019), which postulates that pest regulation will be only slightly improved by flower strips in a homogeneous landscape of field crops where natural enemies are too scarce, as well as in a very complex landscape where natural enemies and their vital resources are already very abundant, thereby negating the added value of a new AEI. However, the results documented in recent literature do not always confirm this theoretical expectation (Albrecht *et al.*, 2020; Hoffmann *et al.*, 2021).

The results of the analysis of the effect of climate on the effectiveness of flower strips (see previous section), although very general, suggest that the composition of these strips could be adapted to the different beet growing regions of mainland France. In oceanic to temperate climates, preference should be given to frost tolerant species that flower at the end of winter, in order to encourage the early arrival and activity of parasitoids near the beet crops. In continental climates, autumn-flowering species forming



a persistent winter cover and offering a winter diapause site for the parasitoids would be recommended. The mix could be completed by spring-flowering species providing resources (nectar and pollen) for natural enemies at the start of the growing season.

In order to document the combined effect of landscape characteristics and climatic context on the effectiveness of flower strips in regulating pests, we are currently working with the ITB (French sugar beet technical Institute) to monitor a series of fields with flower strips in a network of pilot farms spread across the production area.

#### General conclusion

The semi-quantitative analysis of 58 studies on the effect of flower strips on the regulation of pest populations by their natural enemies shows that these AEIs are promising for improving the biological control of pests in field crops. It confirms the results of a recent quantitative study which estimated a 16% improvement in the regulation service, all variables taken together (n = 18 studies; Albrecht *et al.*, 2020). In the case of aphid vectors of beet yellows, this finding remains to be confirmed in the field. Flower strips are agro-ecological features that should be combined with other methods in crop protection strategies, based for example on manipulating aphid behaviour (repellent and attractive scents or visual confusion through mulching) or developing resistant varieties or curative solutions (e.g. aphicides). These strategies have yet to be fully developed on the basis of the work carried out in the various PNRI projects, in order to provide credible alternatives to neonicotinoids.

The resources provided for natural enemies can be adjusted by adapting the composition of the flowering mixtures to the species of parasitoids and predators found locally, depending on the target pest (in this case aphids that carry yellows) and the different landscape and climatic conditions to be covered. In addition, the proposed mixtures must have solid technical references for evaluation and implementation, and be able to be installed with good reliability in variable climatic and soil conditions, using seeds that are available and easy to use. These mixtures can also be used to regulate several pests, particularly when they are perennial and border a field where a succession of crops is grown (e.g. beet/cereal/oilseed rape). Lastly, on a regional scale, a reorganisation of the agricultural area to integrate a functional network of AEIs (sufficient area and satisfactory connectivity), taking into account the extent of the expected effects (e.g. distance of effect of a flower strip) should be recommended. This approach requires a consensus between stakeholders and a shared desire for collective implementation. It could be based on European nature restoration policies (included in the Green Pact for Europe), which encourage the installation of "high biological diversity" landscape elements in agro-ecosystems.

#### **Ethics**

The authors declare that the experiments were carried out in compliance with the applicable national regulations.

#### Declaration on the availability of data and models

The data supporting the results presented in this paper are available on request from the author of the paper.

## Declaration on Generative Artificial Intelligence and Artificial Intelligence Assisted Technologies in the Drafting Process.

The authors have used artificial intelligence-assisted technologies to translate from French to English.

#### **Author ORCIDs**



Joan van Baaren: ORCID: 0000-0002-8552-9645

Kevin Tougeron: 0000-0003-4897-3787

Anne Le Ralec: ORCID : 0000-0001-9570-231X Yann Tricault : ORCID: 0000-0002-7778-5163

Cécile Le Lann: 0000-0002-3949-4066 Manuel Plantegenest : 0000-0002-7964-1374 Ronan Marrec : 0000-0003-1607-4939

#### **Authors' contributions**

Ludovic Lagneau made the bibliographic research and the first draft of the tables and analyses. Joan van Baaren and Yann Tricault wrote the first draft of the paper. All co-authors revised the manuscript.

#### **Declaration of interest**

The authors declare that they do not work for, advise, own shares in, or receive funds from any organisation that could benefit from this paper, and declare no affiliation other than those listed at the beginning of the paper.

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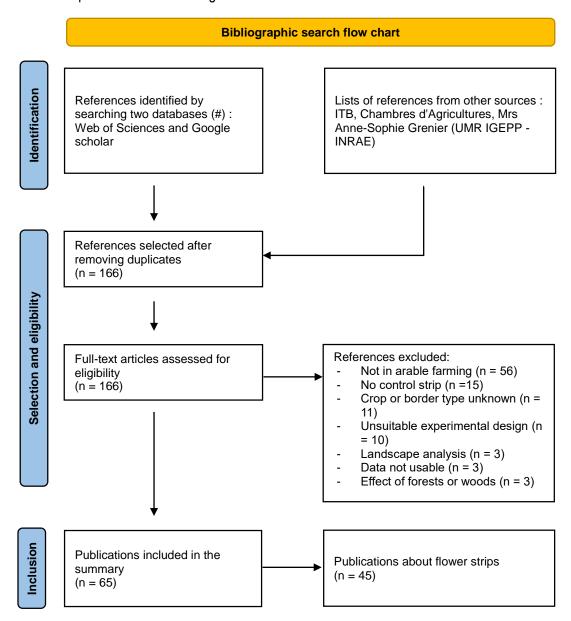
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#### **Appendix 1**: Bibliographical research method and list of references

A literature search on the effect of AEIs on the biological control of pests was carried out to identify all the publications available on the period 1993 - 2021. The method used to extract all the usable works devoted to flower strips is summarised in Figure A.



#) Search equation used: (flower OR flowers OR "flower strip\*" OR "wild flowers" OR wildflower\* OR "agrienvironment scheme\*" OR grass OR "grass margin\*" OR grassy OR "grassy margin\*" OR edges OR hedgerow\* OR "wood margin\*" OR "woody margin\*") AND (control OR biocontrol OR "pest control" OR pest OR virus) AND (predator OR predators OR parasitoid\* OR "natural enem\*" OR "beneficial insect\*") AND (cereal OR potato OR "oilseed rape" OR "oil seed rape" OR "faba bean" OR fababean OR pea OR cabbage OR corn OR wheat OR soybean OR beet OR barley)

Figure A. Bibliographic research method. Adapted from : Page M.J., McKenzie J.E., Bossuyt P.M., Boutron I., Hoffmann T.C., Mulrow C.D., *et al.* The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021; 372:n71. doi: 10.1136/bmj.n71.



Information on the characteristics of the AEIs (size, age and composition) and the landscape and climatic contexts was extracted from each selected publication, as were the effects of the AEIs on the various variables of interest:

- -Crop yields
- -Direct and indirect damage to crops caused by pests (in particular the symptomatic expression of viral diseases transmitted by insect vectors)
  - -The abundance of pests (aphids in particular) in crops
- -Abundance and species richness of natural enemies (predators and parasitoids) in crops and/or AEIs
  - -Parasitism and predation rates at the expense of pests

The semi-quantitative analysis of this corpus consisted in counting the positive, negative or neutral effects (in the agronomic sense, a positive effect being favourable to the crop) among all the studies listed, distinguishing between the different variables of interest.

A second literature search was carried out to list the natural enemies of the aphids that transmit *Myzus* persicae and *Aphis fabae* yellows mentioned in the scientific literature. To do this, the following query was submitted to the Google Scholar and Web of Science databases:

Myzus persicae OR Aphis fabae AND ("natural enem\*" OR predator OR predators OR parasitoid OR parasitoids)

Only European studies were analysed, with no restrictions on the crop studied this time.

The full list of references used in this article, including the 45 publications on the role of flower strips in regulating aphids, the 32 publications used to identify their natural enemies and the 65 publications on the effect of climate, is available at this address: https://doi.org/10.5281/zenodo.7782707

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