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ORIGINAL ARTICLE

Enhancing arthropod communities through plant diversified edge of kale cultivation

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Abstract - Plant diversification can increase organism abundance while reducing phytophagous insect activity in agricultural crops. We assessed arthropod diversity in fava beans (*Vicia faba*), fennel (*Foeniculum vulgare*), and marigolds (*Tagetes patula*) along the periphery of a kale plantation and examined the influence of their proximity to the kale cultivation. The study took place at the Centro Estadual de Diagnóstico e Pesquisa Florestal, Santa Maria, RS, Brazil. We collected plant samples from the periphery and cultivated kale plants at three distances from the edge: within two meters, 10 to 12 meters, and 20 to 22 meters. We recorded the number and biomass of marketable leaves from three harvests. A total of 618 arthropods were collected from plants along the periphery: Insecta (589), Arachnida (20), and Entognatha (9). Hemiptera was the most prevalent order (49.84%). Kale plants near the periphery experienced less predation from leaf beetles. Plants along the periphery hosted important predator groups as well as phytophages that can serve as alternative prey. Diversifying the periphery with fennel, fava bean, and marigolds shows promise as a strategy to enhance the arthropod community in kale cultivation, thereby acting as a conservative biological control.

Keywords: Conservative biological control. *Tagetes patula*. *Foeniculum vulgare*. *Vicia faba*. *Brassica oleraceae*.

Melhorando as comunidades de artrópodes através da diversificação vegetal da bordadura do cultivo de couve

Resumo - A diversificação vegetal em ambientes agrícolas pode favorecer a abundância de organismos, e reduzir a ação dos insetos fitófagos nos cultivos. O estudo teve como objetivo avaliar a diversidade de artrópodes em fava (*Vicia faba*), funcho (*Foeniculum vulgare*) e cravo-de-defunto (*Tagetes patula*) mantidos na bordadura do plantio de couve, bem como o impacto do distanciamento destas plantas no cultivo. O ensaio foi conduzido no Centro Estadual de Diagnóstico e Pesquisa Florestal, Santa Maria, RS. Foram amostradas plantas da bordadura, e plantas de couve distanciando da bordadura em até dois metros, de 10 a 12 m, e de 20 a 22 m. Registrou-se o número e a massa de folhas comercializáveis em três colheitas. Foram coletados 618 artrópodes nas plantas da bordadura, distribuídos em Insecta (589), Arachnida (20) e Entognatha (9). Hemiptera foi a ordem mais representativa (49,84 %). Plantas de couve próximas da bordadura sofreram menor predação por coleópteros desfolhadores. As plantas da bordadura abrigaram fitófagos, que podem atuar como presas alternativas, e importantes grupos de predadores. Neste sentido, a diversificação da bordadura com funcho, fava e cravo-de-defunto pode ser uma estratégia promissora no incremento da comunidade de artrópodes na área de cultivo da couve, visando o controle biológico conservativo neste agrossistema.

Palavras-chave: Controle biológico conservativo. *Tagetes patula*. *Foeniculum vulgare*. *Vicia faba*. *Brassica oleraceae*.

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Introduction

Plants belonging to the Brassicaceae family are significant agricultural crops due to their nutritional, commercial, and economic value, mainly because of their relatively low production costs (FILGUEIRA, 2000; HOLTZ *et al*., 2015). Kale (*Brassica oleraceae* L.) is a crop with a short cycle that could be even more productive if it were not frequently devastated by phytophagous species, such as the diamondback moth (*Plutella xylostella* L., Lepidoptera: Plutellidae), kale leafworm (*Ascia monuste orseis*, Latreille, Lepidoptera, Pieridae), cabbage looper (*Trichoplusia ni* Hübner, Lepidoptera, Noctuidae)), cucurbit beetle (*Diabrotica speciosa* (Germar), Coleoptera: Chromelidae), aphids (*Brevicoryne brassicae* L. and *Myzus persicae* Sulzer, Hemiptera: Aphididae), and whitefly (*Bemisia tabaci* (Gennadius), Hemiptera: Aleyrodidae) (HOLTZ *et al*., 2015).

Conservative biological control offers a means to reduce the number of herbivorous insects by managing agroecosystems to preserve and enhance natural enemies, such as parasitoids, predators, and pathogens (FONTES *et al*., 2020). Several studies have demonstrated that habitat manipulation, such as incorporating plant species in the form of strips, edges, corridors, polycultures, or maintaining weeds, can decrease herbivorous insect populations and/or increase the community of beneficial insects (AMARAL *et al*., 2013; BALZAN, 2017; BOETZL *et al*., 2019; BRENNAN, 2016; HATA *et al*., 2016; QUINN *et al*., 2017; RIBEIRO; GONTIJO, 2017; SCHULZ-KESTING *et al*., 2021).

Plant diversification in agricultural environments can act as physical and chemical barriers to pests. It hinders the localization and colonization of the host crop by pests, provides microclimates and favorable environments for natural enemies, and contributes to the repository of prey and alternative hosts that can attract and maintain predators and parasitoids even before crops are installed (BARBOSA *et al*., 2011; FONTES *et al*., 2020). The nectar offered by plants serves as a source of carbohydrates, lipids, amino acids, phenols, alkaloids, and volatile organic compounds (GONZÁLEZ-TEUBER; HEIL, 2009). Some of these components are essential for the survival of adult parasitoids and serve as a complementary dietary resource for many predators, leading to increased longevity and/or fecundity in the presence of flowers (BATISTA *et al*., 2017; GÉNEAU *et al*., 2012; RUSSELL, 2015; TOGNI *et al*., 2016). Certain species such as *Vicia faba* L. (KWOK; LAIRD, 2012), *Inga subnuda* subsp. *luschnathiana* (Benth.) T.D. Penn. (REZENDE *et al*., 2014), *Senna rugosa* (G.Don) HS Irwin-Beneby (Fabaceae), and *Eryotheca grasilipes* (K. Schum) A. Robyns (Malvaceae) have extrafloral nectaries, which means that nectar is present in plant organs other than the flowers, making them attractive to insects even during the vegetative phase (PIRES, 2015).

Plants of the Apiaceae family are known to attract various groups of insects due to the shallow corollas of their flowers, which provide exposed and easily accessible nectaries (LOVEI *et al*., 1993; TOOKER; HANKS, 2000). Predatory ladybugs have been observed in kale when it is intercropped with Apiaceae plants such as fennel (*Foeniculum vulgare*) and dill (*Anethum graveolens*) (LIXA *et al*., 2010). In laboratory studies, the presence of coriander flowers (*Coriandrum sativum*) significantly increased the survival of the coccinellid *Cycloneda sanguinea* L. (TOGNI *et al*., 2016). In the Asteraceae family, species of the *Tagetes* genus are utilized in organic gardens to enhance the populations of natural enemies and their alternative prey (HARO,

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2014; SOUZA *et al*., 2019). This genus contains secondary metabolites in its leaves and flowers that are capable of pest control in agricultural crops (DARDOURI *et al*., 2017; SALINAS-SÁNCHEZ *et al*., 2012).

The selection of plants for diversifying cultivation must take into consideration the resources they offer, as well as the diversity of beneficial insects and herbivores they can attract (ALTIERI *et al*., 2003). Proper selection can help avoid undesirable species in the target crop. Additionally, determining a specific distance from the crop of interest is important, as there may be a reduction in the abundance of certain insect groups in locations farther away from the diversification (BOETZ *et al*., 2019; BRENNAN, 2016; POLLIER *et al*., 2018; SILVEIRA *et al*., 2009).

Our study aimed to evaluate the diversity of arthropods present in marigolds, fennel, and fava beans maintained at the edge of kale cultivation. Additionally, we assessed the impact of distancing on the presence of pest insects, damage, and crop productivity.

Materials and Methods

The study area consisted of five beds, with each bed containing 100 plants arranged in two rows. The spacing between plants was 0.5 m, and the spacing between rows was 1.5 m. Fertilization was conducted using 162 g/m2 of NPK 10-18-20, and acidity correction was performed with 750 g/m2 of dolomitic limestone. Manual irrigation was carried out as required. Prior to planting kale, 60 seedlings of *Tagetes patula* (Asteraceae) were planted at the distal edge of the beds, distributed in four rows. Additionally, 30 plants of *Foeniculum vulgare* (Apiaceae) were distributed in two rows, and 50 plants of *Vicia faba* (Fabaceae) were placed in three rows (Fig. 1). The kale seedlings were transplanted on June 21st, 2018, while F. vulgare and *F. faba* were in the vegetative stage, and some *T. patula* plants already had flowers. The experimental design employed in this study is novel.

The kale plants were evaluated at three different distances from the edge plants: 1) up to two meters; 2) from 10 to 12 meters; 3) from 20 to 22 meters. Each group consisted of 50 kale plants (Fig. 1). Every week, 10 plants (two per bed) were randomly selected, gently shaken into a plastic bag, and inspected. The number of leaves, presence of insect damage, and measurements of the length and width of the largest leaf were recorded. Additionally, 25 plants of each species were shaken onto a tray (43.5 cm x 29.6 cm x 7.5 cm) containing water and neutral detergent. These weekly samplings were conducted from July to November 2018, totaling 13 sampling occasions. The sampling period was from 9 am to 12 pm. Each bed was considered a subplot within its respective treatment, serving as a repetition for statistical analysis. All collected organisms were preserved in 70% alcohol for subsequent taxonomic identification.

Throughout the study, three kale harvests were conducted at 80, 125, and 176 days after transplanting the seedlings into the field. On each occasion, the leaves produced by all 150 plants in the plot were harvested. The leaves from each plant were categorized as marketable (when they exceeded 25 cm in length and had less than 50% perforation and yellowing) or non-marketable. The number of leaves and the fresh mass of each group were counted and measured.

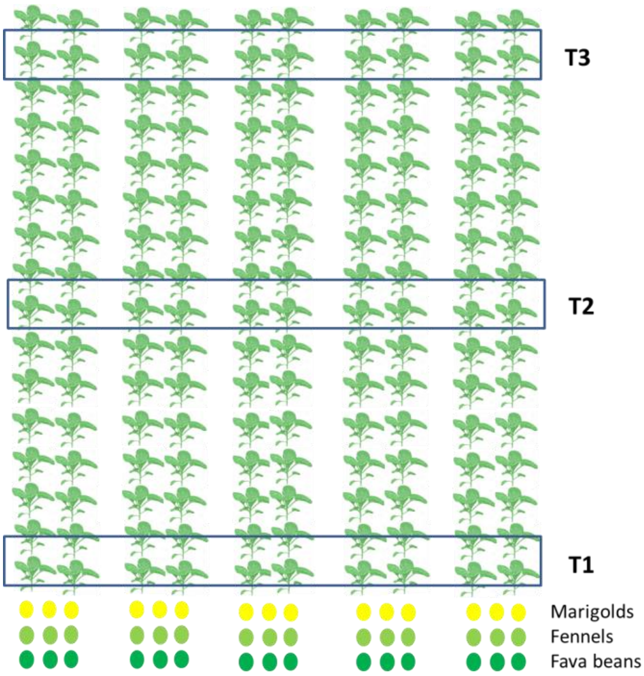

Figure 1. Experimental layout of the cabbage crop area incorporating marigolds, fennel, and fava beans on the edge. The treatments employed in the study were indicated as T1) up to two meters; T2) from 10 to 12 meters; T3) from 20 to 22 meters from the edge. Location: Santa Maria, RS, Brazil.

The following parameters were compared among the plant distance ranges: mean abundance and mean number of plants with arthropods, phytophagous insects (*Plutella xylostella*, *Diabrotica speciosa*, and aphids), mean number of leaves with damage caused by *P. xylostella* or leaf beetles, length (cm) and width (cm) of the largest leaf of the sampled plant, and mean mass and number of marketable leaves per plant. The means of each evaluated parameter were compared among treatments using ANOVA, followed by Tukey's test at a significance level of 5%. All statistical analyses were performed using the software BioEstat 5.0 (AYRES *et al*., 2007).

Results and Discussion

Arthropods present in the edge composed of *Vicia faba*, *Foeniculum vulgare*, and *Tagetes patula*.

A total of 618 specimens of the phylum Arthropoda were collected, including 589 insects (Insecta), 20 spiders (Arachnida), and nine springtails (Entognatha). In the Insecta Class, six orders were represented. Hemiptera was the most abundant order, followed by Diptera and Coleoptera (Table 1).

Among the order Hemiptera, three suborders were represented in different proportions: Auchenorrhyncha (leafhoppers) with 48.05%, Sternorrhyncha (aphids) with 41.55%, and Heteroptera (bugs) with 9.74%. Aphids were the most abundant hemipterans in fava bean (48 individuals) and fennel (68 individuals), while only five individuals were sampled in marigolds. Aphids are typically found in plant shoots due to their higher nitrogen concentration (GRAZIA *et al*., 2012). We observed colonies of aphids at

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the apex of fava bean plants. Leafhoppers were collected in large numbers from fava bean (45 individuals), fennel (52 individuals), and marigolds (31 individuals), and they were mainly represented by the Cicadellidae family. Cicadellids constitute the largest group within Auchenorrhyncha, and species from this group are cosmopolitan and infest numerous plant species (BALDIN; FUJIHARA, 2016).

Table 1. Total and relative abundance of arthropod orders sampled from *Tagetes patula* (marigold) (Asteraceae), *Foeniculum vulgare* (fennel) (Apiaceae), and *Vicia faba* (fava bean) (Fabaceae), cultivated in the kale edge. Sampling from July to November 2018, in Santa Maria, RS, Brazil.

	Fava bean	**Fennel**	**Marigold**	**Total abundance**	**Relative abundance (%)**
Arachnida					
Araneae	9	9	2	20	3.24
Entognatha					
Collembola	1	3	5	9	1.45
Insecta					
Coleoptera	30	21	14	65	10.72
Diptera	22	83	18	123	19.90
Hemiptera	129	133	46	308	49.84
Hymenoptera	36	8	5	49	7.93
Lepidoptera	2	15	7	24	3.88
Neuroptera	1	2	1	4	0.65
Thysanoptera	2	2	12	16	2.58
Total	232	276	110	618	
(%)	37.54	44.66	17.80		

Diptera was the second most abundant order in the study. Diptera is a group that includes representatives with varied habits, including pollinators, parasitoids, and predators (CARVALHO *et al*., 2012). Among the predatory species within the larval stage, the Syrphidae family was observed in large numbers in fennel inflorescences (KOPTA *et al*., 2012). Despite fennel being in the vegetative stage, we collected a higher abundance of Diptera on this plant. It is possible that the insects were attracted by volatiles released by fennel, which mainly consists of compounds such as anethole (trans-anethole), estragole, fenchone, p-anisaldehyde, and cis-anethole (DIÄAZ-MAROTO *et al*., 2005). For example, anethole can be attractive to dipterans such as *Plecia nearctica* Hardy (Bibionidae) (CHERRY, 1998).

Coleoptera, on the other hand, exhibits a variety of feeding habits, including many predatory families. Most adults and larvae of ladybirds (Coccinellidae) are active predators, primarily feeding on aphids and scales, although some species specialize in consuming small arthropods such as mites, ants, and other beetles (CASARI; IDE, 2012). In our study, we only collected ladybirds on fennel and fava bean plants, which can be attributed to the high densities of aphids on these plants. This finding is consistent with the results of Kopta *et al*. (2012), who observed ladybirds feeding on aphids on fava bean flowers and fennel inflorescences.

The phytophagous beetle *Diabrotica speciosa* (Chrysomelidae) is a significant pest in kale cultivation (HOLTZ *et al*., 2015). Although we sampled only 24 individuals of this species throughout the study, 19 of them were collected from fava bean plants. Fava beans may be one of the preferred plant families for *D. speciosa*. Despite being polyphagous, this species is considered a pest in agricultural crops of the Fabaceae, Solanaceae, Curcubitaceae, and Poaceae families, which have high economic value (CATAPAN *et al*., 2018).

More than half of the collected Hymenoptera were from the Formicidae family (55.1%), and almost all were collected from fava beans. Ants preferentially forage on plants with extrafloral nectaries; they are even more attracted to plants damaged by phytophagous since their damage induces the production of more nectaries (KWOK and LAIRD, 2012). Aphids may have been responsible for the presence of ants since both families, Formicidae and Aphididae, were sampled in the same period. Some species of ants also feed on the sugary liquid released by aphids. They start to live in cooperation in which ants receive food from the aphids while offering them protection (GRAZIA *et al*., 2012). Other Hymenoptera, such as bees of the *Apis* genus and predatory wasps, also composed the samples though in lower numbers.

The order Neuroptera occurred in a reduced number, being represented by the Chrysopidae family. Only larvae were collected, which may have been due to the sampling method that is not suitable for capturing adults. Green lacewing larvae have great potential as pest control since they are voracious insect predators that prey on various groups, such as scale, aphids, thrips, whiteflies, leafhoppers, mites, small caterpillars, and eggs and larvae of pests (CAMARGO, 2016). Adults of some species, such as those from the genus *Chrysoperla*, feed only on sugary substances and pollen (STELZI and DEVETAK, 1999), being attracted to flowery locations.

Thrips (Thysanoptera) were collected in greater numbers on marigold plants, possibly due to chemical compounds present in plants of the genus *Tagetes*, such as (E)-b-Farnesene, which attract thrips species (HARO, 2014; KOSCHIER *et al*., 2000). Flower strips with *T. patula* from the edge of melon plantations (*Cucumis melo* - Curcubitaceae) harbored non-pest phytophagous thrips (PERES, 2007). Thus, the presence of thrips is promising since they constitute an alternative prey for predators.

Regarding seasonality, the greatest abundance of individuals (82.36%) was recorded from the end of August (Fig. 2). Temperatures begin to rise in this period, providing favorable conditions for the growth, reproduction, and dispersal of insects in general (SPEIGHT *et al*., 1999). Fava bean and marigold plants reached their highest flowering peak after the end of August, which remained throughout the spring, possibly contributing to the attraction of organisms (Fig. 3). The density of insects present in the fennel may have fluctuated more due to abiotic factors than to the plant's phenology, as the plants were in the vegetative stage during the entire evaluation period.

Analysis of kale plants present in the three distance gradients

A total of 568 arthropods were sampled in the kale plants. They encompassed Lepidoptera (83.27 %), Coleoptera (6.86 %), Hemiptera (4.75 %), Diptera (2.82 %), Hymenoptera (1.06 %), and Araneae (1.06 %). Lepidoptera was the most abundant, exclusively represented by the species *P. xylostella*.

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Kale plots kept within two meters of the edge had a lower number of individuals, a lower number of plants with *D. speciosa* ($p < 0.05$), and a lower number of leaves damaged by leaf beetles than plants 20 to 22 meters away from the edge ($p < 0.01$) (Table 2).

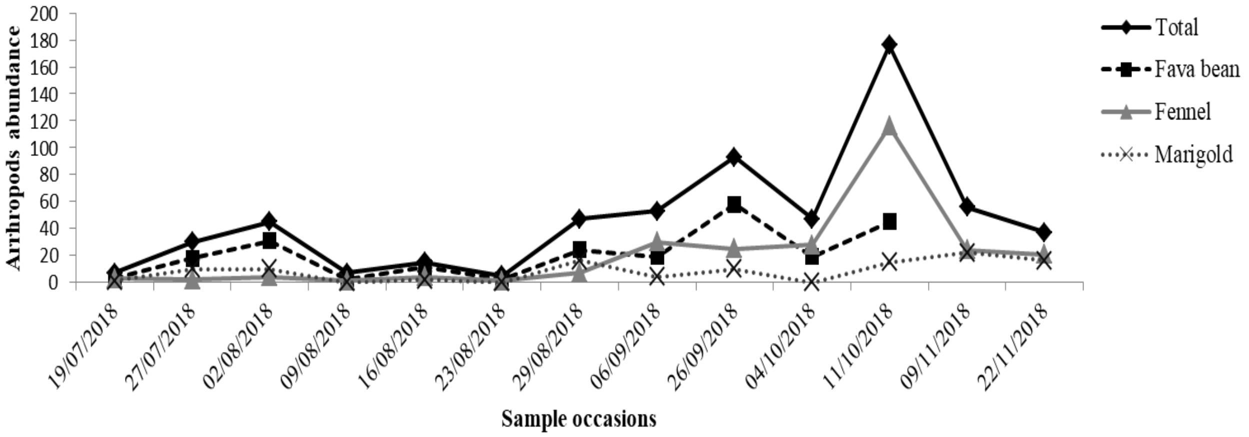
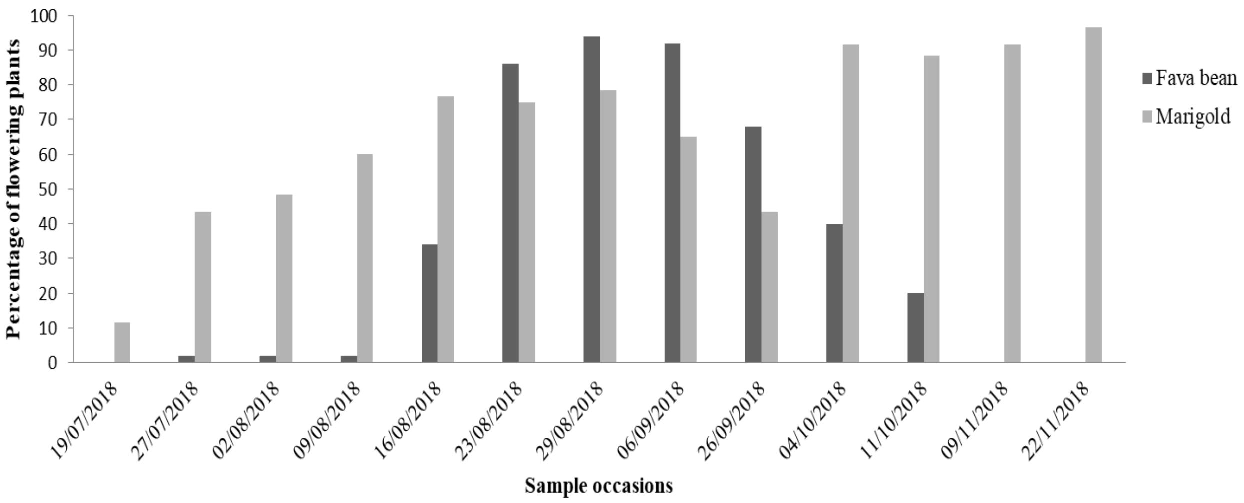


Figure 2. Abundance of arthropods in plants present in the edge of a kale cultivation. Total abundance in fava bean (*Vicia faba*), fennel (*Foeniculum vulgare*), and marigold (*Tagetes patula*) plants. Sampling from July to November 2018, in Santa Maria, RS, Brazil.



ta 3. Percentage of fava bean (*Vicia faba*) and marigold (*Tagetes patula*) plants flowering during the sampling period, from July to November 2018, in Santa Maria, RS, Brazil.

Although there was not a significant number of *D. speciosa* throughout the study, repellent compounds present in some plant species at the edge may have influenced the occurrence and distribution of chrysomelids in kale plants. Among the plants at the edge, marigolds have the potential to repel several species of insects (DARDOURI *et al*., 2017; LOVATTO *et al*., 2013; SALINAS-SÁNCHEZ *et al*., 2012; SIGNORINI *et al*., 2016). Laboratory tests showed that leaf discs soaked with *Tagetes minuta* extract led to the mortality of *D.*

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speciosa (TRECHA, 2018). In a study using intercropping of aromatic plants in a pear (*Pyrus pyrifolia*) orchard, there was a reduction in the abundance of Scarabaeidae pests and an increase in the abundance of parasitoids in plots intercropped with *T. patula* (TANG *et al.*, 2013). According to the authors, intercropping with aromatic plants changes the interactions between insect pest populations and populations of their natural enemies.

Table 2. Values are the mean total abundance of arthropods, mean number of leaves (damaged by *P. xylostella* and by leaf beetles), size of the largest leaf of the sampled plant (length and width in cm), and marketable leaves parameters (average number and mass (g) of per kale plant), in a cultivation of kale located within 2 m, from 10 to 12 m, and from 20 to 22 m away from an edge with cultivated marigold (*Tagetes patula*), fennel (*Foeniculum vulgare*), and fava bean (*Vicia faba*) plants, from July to December 2018.

Average value per subplot	**0 to 2 m**	**10 to 12 m**	**20 to 22 m**
Abundance			
Arthropods	44.4	29.4	39.8
Phytophagous insects	34.2	28.2	38.8
Plutella xylostella	30.2	24.4	30.4
Diabrotica speciosa	0.6 b *	1.8 ab	3 a
Aphids	4	2.3	3.6
Number of infested plants			
Arthropods	15.2	17	22
Phytophagous insects	14.2	16	18.8
Plutella xylostella	12.8	12.8	14.4
Diabrotica speciosa	0.4 b	1.6 ab	2.4 a
Aphids	1	2	2.3
Number of damaged leaves by herbivore			
Plutella xylostella	244.6	259.4	276.2
Leaf beeatles	40.6 b	64 ab	72.8 a
Size of the largest leaf of the plant (cm)			
Length	28.49	28.43	28.26
Width	17.74	17.53	17.38
Marketable leaves per plant **			
Number	16.17	15.62	14.87
Mass (g)	424.21	421.3	394.76

* Means followed by distinct letters in the line differ according to Tukey's test at 0.05 of significance.
** Average values of the three harvests.

Marigolds may also exhibit repellency to aphids, regardless of their distance. Their abundance decreased during sampling, with a total of 27 individuals found in the kale. According to Ben Issa *et al.* (2016), *T. patula* plants release numerous volatile organic compounds that affect aphid performance. Over 50 compounds emitted by *T. patula* have been identified (DARDOURI *et al.*, 2017). In this study, the

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olfactometer did not significantly affect aphids' orientation behavior towards the volatile compounds of *T. patula*. However, a lower incidence of aphids occurred in the field when pepper (*Capsicum annuum* L.) cultivation was intercropped with marigold plants. The authors suggested that marigold compounds might modify the host plant's volatile emission, indirectly influencing aphid behavior.

Other variables evaluated in this study did not show significant differences among the three distances from the edge (Table 2). It is possible that the plants at the edge did not affect the other evaluated groups. Another hypothesis is that the maximum evaluated distance was not far enough to generate any difference along the gradient. Silveira *et al*. (2009) observed differences in the abundance of organisms in onion (*Allium cepa* L.) when comparing distances of five and 30 meters from the *Tagetes erecta* L. strip in the margin vegetation. Perhaps we would find differences in the abundance of other groups if a distance greater than 22 meters was evaluated.

The diamondback moth comprised over 80% of all collected organisms, and its abundance remained constant throughout the study. The constant presence of *P. xylostella* and the similarity in the number of leaves with caterpillar damage at the three distances may have influenced the final yield. Even though the highest averages of leaf number and mass in the closest range (up to 2 meters) were recorded in the first harvest, there was no significant difference between distances ($p \geq 0.05$) (Table 2). According to Czepak *et al*. (2005), the cruciferous moth is capable of considerably reducing kale productivity, damaging up to 95% of it depending on the region and time.

The plants evaluated in this study exhibited potential for use at the edge of kale cultivation. Despite the presence of numerous phytophagous insects, none of the most significant groups were major pests in kale cultivation. Aphids were the most abundant insects found on fava bean and fennel plants. However, the aphids observed as pests on these plants are distinct from those plaguing brassica crops, which are primarily *Brevicoryne brassicae* (L.) and *Myzus persicae* (Sulzer) (HOLTZ *et al*., 2015). The edge plants can serve as reservoirs for trapped phytophagous insects, a crucial characteristic for attracting and maintaining predators in this edge area before kale is established. Marigolds had the lowest incidence of insects, despite flowering throughout the entire sampling period. Nonetheless, it played a crucial role in the entomofauna's composition, providing shelters for species like thrips, which had limited occurrence on other plants.

Even though fennel plants remained in the vegetative phase throughout the study, they harbored the greatest abundance of insects. Several studies highlight fennel's importance in attracting floral visitors, including natural enemies that rely on pollen as part of their diet and other polyphagous insects (COLLEY; LUNA, 2000; KOPTA *et al*., 2012; LIXA *et al*., 2010; SKALDINA, 2020). Our findings demonstrate that even before flowering, this aromatic plant already attracts essential arthropod groups to the cultivation area.

Maintaining a diversified edge with plants from different families and ecological roles increased the biodiversity of arthropods within the cultivation. Further studies are needed to propose alternative plant arrangements, elucidate the interactions between organisms and plants, and promote the conservative biological control of phytophagous species of economic importance in this agroecosystem.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any potential conflicts of interest.

Ethical Statements

The authors confirm that the ethical guidelines adopted by the journal were followed by this work, and all authors agree with the submission, content and transfer of the publication rights of the article to the journal. They also declare that the work has not been previously published nor is it being considered for publication in another journal.

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