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ORIGINAL ARTICLE

Bioaccumulation of pesticides in carabid beetles in a vineyard and olive grove under integrated pest management*

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Abstract. Intensive use of pesticides is among the main drivers of biodiversity loss, especially of insects. Here, field concentrations of chemical synthetic pesticides were measured in soil and carabid beetles in a vineyard (VP) and olive grove (OP), in two consecutive years. The aim was to determine if active ingredients in pesticides applied in the field accumulate in carabids and how this correlates with treatment intensity. Carabids and soil samples were collected at a vineyard and olive grove in Zadar County in Croatia, soil in 2018 and 2019 and carabids in 2019. Both were under integrated pest management (IPM), with a total of 34 pesticides applied, between January and August in the two years of this study. Using LC-MS/MS, a broad range of pesticides, mainly fungicides, was detected in the soil and carabids. In soil samples, boscalid (0.047 mg/kg), mandipropamid (0.08 mg/kg), fluopyram (0.09 mg/kg), cyprodinil (0.09 mg/kg) and tebuconazole (0.13 mg/kg) were detected in the highest amounts. In addition, nine substances were detected in carabids, with valiphenalate (0.048 mg/kg), difenoconazole (0.051 mg/kg) and azoxystrobin (0.064 mg/kg) in the highest concentrations. Bioaccumulation factor (BAF) indicated the accumulation of valiphenalate, metalaxyl-M, spiroxamine and difenoconazole in carabids. Data measured directly in the field revealed the accumulation of pesticides in carabids, which indicates they could be good bioindicators in IPM and contribute to a better understanding of the distribution of pesticides in Mediterranean agroecosystems.

INTRODUCTION

Wide and frequent use of pesticides is considered to be one of the main drivers of loss of biodiversity, especially of invertebrates (Dudley et al., 2017; Homburg et al., 2019; Sanchez-Bayo & Wyckhuysb, 2019; Ali et al., 2021; Andrade et al., 2021; Tang et al., 2021) and results in many negative effects on non-target species and ecosystems (Whitehorn et al., 2012; Goulson, 2013). Due to the adverse effects on pollinators, especially bees, the European Commission relatively recently restricted the use of neonicotinoids to greenhouses and banned their use in outdoor areas in 2018 (EU Commission, 2018). Basley & Goulson (2017) report negative effects of clothianidin on food intake in earthworms and also confirm that doses used in the fields increase earthworm mortality. In addition, to improving soil fertility, structure and organic matter content earthworms are important food for many predatory arthropods like carabids (e.g. Šerić Jelaska & Symondson 2016). After application in the field, pesticides can accumulate in soil and non-target animals, either passing through their skin or being absorbed after ingestion (Lushchak et al., 2018). Non-target invertebrates, among which there are many beneficial species, providing ecosystem services such as biocontrol and pollination, accumulate pesticides not only by direct exposure but also from food they consume (Cloyd & Bethkeb, 2011; Goulson, 2013; Pisa et al., 2014; Bonmatin et al., 2015; Botías et al., 2016). There is, however, insufficient data on the distribution of pesticides, effects on non-target species and ecological interactions in the field (Wood & Goulson, 2017). In the case of predators, experiments mostly estimate their abundance in the field post application and little is known about their accumulation of pesticides and transfer to other trophic levels. This is especially the case in the Mediterranean area with ecosystems that are particularly vulnerable to the climate and anthropogenic changes resulting in the loss of biodiversity (Newbold et al., 2020).

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Carabids are among the most abundant arthropods in agroecosystem and they are important in pest and weed control (Lundgren, 2009; Bohan et al., 2011), as they feed on slugs (Symondson, 1997; Hatteland et al., 2010; Šerić Jelaska et al., 2014a, b), moths (Suenaga & Hamahura, 1998; Šerić Jelaska et al., 2014b), aphids (Bryan & Wratten, 1984), dipteran larvae (Kromp, 1999) and weed seeds (Saska, 2004; Bohan et al., 2011; Petit & Bohan, 2018). Previous studies, in situ and in vitro, showed that exposure to pesticides can cause lethal and sublethal effects in various non-target arthropods, including carabids (e.g. Tooming et al., 2017; Ivanković Tatalović et al., 2023). Carabids can be exposed to pesticides by direct contact with their body surface (Yao et al., 2015), consumption of spray droplets (Kunkel et al., 2001) or ingestion of contaminated food (Prasifka et al., 2008, Douglas et al., 2014). There is still, however, little information on the distribution and accumulation of pesticide in field conditions, acute and long-term effects of exposure to pesticides and ways of exposure and transmission between trophic levels by carabid beetles is insufficient to predict the endpoints of many chemically synthetized compounds.

Insecticides, such as neonicotinoids, may negatively affect the role of predatory carabids in pest biocontrol (Douglas et al., 2014). Fungicides are the third most important pesticide used worldwide and is higher in wine producing regions, however their toxicity to animals is not sufficiently researched (Zubrod et al., 2019). Despite the fact that fungicides target metabolic pathways characteristic of fungi they are a threat to other organisms, including invertebrates (Zubrod et al., 2019).

Application of herbicides is increasing worldwide, especially in vineyards (Zaller et al., 2018). There is little known about their potential side effects on the soil fauna in vineyards, which is likely to differ from those reported in other crops, like annual crops, because vineyards have been long term and more intensively managed (Zaller et al., 2018). El Jaouhari et al. (2023) suggest that the higher abundance of predatory macrofauna, herbivores and decomposers in organically farmed fields is due to the presence of an abundance of weeds and continuous soil cover when herbicides are not used. Herbivores are directly affected by the suppression of weeds, leading to a decrease in their abundance and thus a decline in predator abundance. Reduction in groundcover plants after treatment with glyphosate affect the activity of ground-dwelling predators (Cruz-Miralles et al., 2022). The abundance of many invertebrates is reduced to varying extents by applications of glyphosate. The biological significance of this effect is limited to shifts in species composition of the flora and structure of habitats (Sullivan & Sullivan, 2003). Glyphosate appears to have no effect on lycosid spiders and only a slight adverse effect on carabid beetles (Michalková & Pekár, 2009). Brust (1990) reports that glyphosate does not have a significant acute or chronic effects on male or female carabid longevity or food consumption, based on one year of exposure to field-rate applications. There are, however, very few studies on their effect on carabids.

Despite an increasing intensification of viticulture and olive production not much is known about the effect of pesticides on their non-target fauna. To contribute to the important issue of conserving biodiversity, a field study was carried out in an agricultural area with vine and olive oil production in Mediterranean Croatia. The aim of this study was to determine pathways of pesticides from soil into wildlife. The concentration of pesticides in soil and carabids was determined over two years in these agricultural areas and the bioaccumulation of pesticides in the soil and carabids and how it was related to treatment intensity was also determined. It was hypothesized that applied pesticides will end up in carabid beetles, and that their active ingredients and/or metabolites will accumulate in their bodies. Though they are not the target of pesticide treatments, a continuous exposure of carabid beetles to pesticides can have an effect on their metabolism and activity and thus negatively affect the ecosystem services they provide. Also, being a prey of insectivores the active ingredients and/or their metabolites will spread further in ecosystems.

MATERIAL AND METHODS

Sites studied

Sampling sites were located near Zadar, in southern Croatia, in an olive grove (OP) in Škabrnja (44°9′49.37″N; 15°44′15.01″W), with a size of 8,000 m^2, and vineyard (VP) in Baštica (44°9′26.21″N; 15°26′3.43″W) 63,000 m^2 in size (Fig. 1), both are under Intergraded Pest Management (IPM). In both, chemically synthesized pesticides were used according to IPM best practice. For each date samples were collected, information on the amount of pesticide applied in 2018 and 2019, were obtained and the amount of active ingredients per hectare per season calculated (Table S1). At each site, 12 areas to be sampled were arranged in a line, 15 m apart, in the centre of the area in order to avoid edge effect.

Pesticide applications and Treatment Index

Table S1 presents the amount of the active ingredients in the pesticides applied over the two years of this study in a vineyard and olive grove, which was 32 and 6, respectively. In addition to copper and sulphur, the substances azoxystrobin and difenoconazole were also applied in both plantations. In both the vineyard and olive grove, copper and sulphur were applied in both years to control fungi or in the case of the olive grove to control insects (sulphur).

The total number of times tractors were used for spraying chemical agents for plant protection in the vineyard was 12 in 2018, and 15 in 2019, respectively. The highest treatment index was recorded for sulphur in the vineyards, whereas pesticides were used once or a maximum of three times during the growing season.

The treatment index for difenoconazole was highest in the olive grove. The application of copper and dimethoate in 2019 was above the legal maximum allowed, due to the fact that the treatment had to be repeated because of rainy weather. The total number of times tractors were used for spraying chemical agents for plant protection in the olive grove was 8 in 2018, and 9 in 2019, respectively.

The maximum number of applications per year was that recommended by the phytosanitary department of the Croatian Ministry of Agriculture for each year up to and including 31st of December.

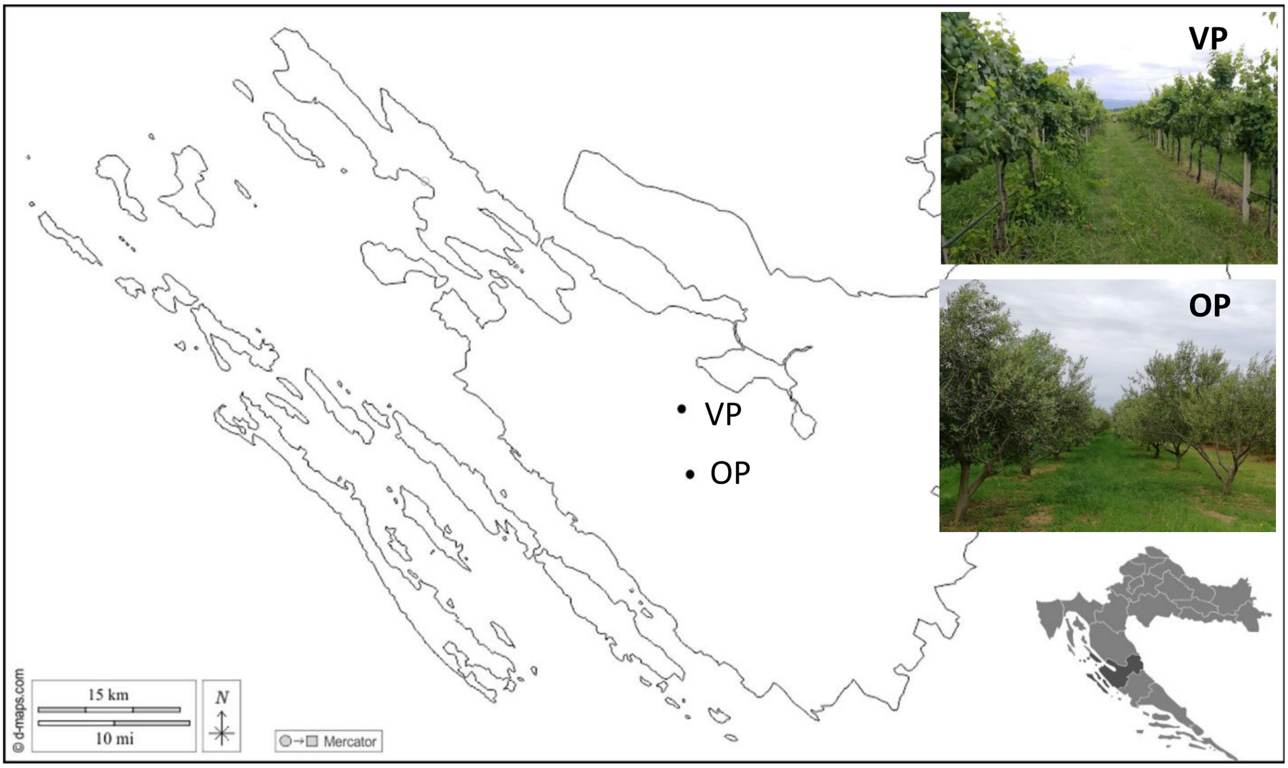

Fig. 1. Sites sampled near Zadar: OP – olive grove (plantation of olive trees) located at Škabrnja and VP vineyard (grape-bearing vines) at Baštica.

Field sampling and laboratory analyses

Soil was collected in 2018 and 2019, and carabid beetles in 2019 during the vegetative season. For pesticide analyses, 12 soil samples (3 L) per site were collected twice, in late spring and autumn, from the top 10 cm of soil at each site. Soil samples were collected in plastic bags and then ground and sieved through a 0.8-mm mesh and stored at room temperature until analysed. Soil pH was determined by adding 10 ml of deionised water to approximately 1 g of dried sample. To measure organic matter, 10 g of soil was burnt at 500°C for four hours. Water holding capacity of the soil was measured as the maximum amount of water retained by soil using Kopecky cylinders, which were weighed empty, then with a soil sample (V = 100 cm^3) collected in the field and again 24 h after absorbing water, then the soil was dried and weighed again. Water capacity is the differences between the weights of the wet and dry soil.

To measure concentration of pesticides in animal tissue, ground beetles (Coleoptera: Carabidae) were collected live using pitfall traps (0.3 L containers) and by hand, in May, July and September in 2019. Pitfall traps contained an attractant (small sponges-soaked in vinegar placed at the bottom of trap). Carabids were placed individually for 24 h in Petri dishes containing moist filter paper to empty their guts, killed at –80°C then weighed and stored in a freezer (–20°C) before chemical analysis. For multispectral chemical analyses, samples were pooled in order to achieve a body mass > 1 g per sample (Table 2).

The multiresidue pesticide analyses were based on multiple mass spectrometry using liquid chromatography (LC-MS/MS), which can identify more than 400 different chemical groups of pesticides (organophosphates, organochlorines, neonicotinoids, etc.). The multiresidue analyses were done by Eurofins Croatiakontrola Ltd., using standard analytical methods HRN EN 15662:2018 (EN 15662:2018) that comply fully with legislation requirements, primarily EU Regulation no. 396/2005, with a detection limit of 0.01 mg/kg. Polar pesticides like glyphosate, and its metabolites were analysed using the method recommended by the EU Reference Laboratory (QuPPe method) using LC-MS/MS. The QuPPe method is based on the extraction of pesticide residues from an homogenized sample using acetonitrile. For pesticide residues from the group of phenoxy carboxylic acids, hydrolysis was used in order to meet the requirements of Regulation (EC) no. 396/2005. Laboratory detection was done using Agilent technologies (Agilent, Santa Clara, CA, USA).

The experiment was carried out before and after the use of neonicotinoids were banned by the EU Commission for outdoor use, as in 2018 neonicotinoids were still being used in the vineyard and in 2019 they were banned.

Data analysis

A broad range of pesticides were detected in the soil and tissue samples and analysed. The concentration of residues was measured in a total of 21 soil samples and 17 samples of carabid beetles (92 individuals were pooled). Bioaccumulation factors (BAFs) for each pesticide were calculated according to formula (1) by dividing mean pesticide concentrations (c) in predator tissue per season per plot by the mean pesticide concentrations in soil.

(1) *AF (active ingredient) = c(active ingredient) carabids / c(soil)*

Pesticide concentration in soil and carabid tissue is presented as mean value with standard deviation (Table 1) and presented in Fig. 2 using Statistica software (TIBCO Statistica™ 14.0.0.).

RESULTS

The multiresidue analyses revealed a total of 18 active substances in soil and animal samples, at concentrations above 0.01 mg/kg per sample in the IPM vineyard and 6 in IPM olive grove.

Table 1. Pesticide residues (mg/kg dry weight) in the soil and carabid beetles collected in the vineyard and olive groove, both under IPM. Results are mean values and standard deviations (SD), for carabids and soil samples, soil acidity, content of organic matter (%) and water capacity.

Active substances	IPM vineyard in Baštica (VP)		IPM olive grove in Škabrnja (OP)	
	Soil (mg/kg, mean value ± SD)	Ground beetles (mg/kg, mean value ± SD)	Soil (mg/kg, mean value ± SD)	Ground beetles (mg/kg, mean value ± SD)
Azoxystrobin	0.012 ± 0.003		0.013 ± 0.002	0.064 ± 0.0003
Boscalid	0.047 ± 0.044			
Chlorantraniliprole	0.012 ± 0.003			
Cyflufenamid	0.011 ± 0.002			
Cyprodinil	0.090 ± 0.064			
Difenoconazole	0.026 ± 0.011	0.0305 ± 0.032	0.041 ± 0.033	0.051 ± 0.014
Dimethoate				0.011
Dimethomorph	0.036 ± 0.016	0.011		0.017
Fenoxycarb				0.031 ± 0.01
Fluopyram	0.0914 ± 0.039			
Iprovalicarb	0.029202			
Mandipropamid	0.0829 ± 0.047		0.013 ± 0.006	
Metalaxyl-M	0.0134 ± 0.003	0.023		
Pyriofenon	0.023	0.007		
Quinoxyfen	0.0167 ± 0.004			
Spiroxamine	0.0265 ± 0.012	0.013 ± 0.013		
Tebuconazole	0.130 ± 0.08			
Thiamethoxam	0.017 ± 0.006			
Triadimenol	0.018 ± 0.007			
Valifenalate	0.023	0.055		
Soil type/preparation	loamy/mulching		red-brown/mulching	
Organic matter %	4.736 ± 0.477		3.600 ± 0.440	
Soil pH (H_2O)	7.522 ± 0.116		7.613 ± 0.064	
Soil water capacity	38.2		31.8	

The highest concentrations recorded in the soil samples collected in the vineyard were for fungicides, such as, tebuconazole, fluopyram, cyprodinil, mandipropamid and boscalid (Table 1). Of the detected pesticides, boscalid was not applied in the vineyard studied in 2018 and 2019 (Fig. 1, Table S1) and even the few years before, but it was applied in a nearby apple orchard in 2018 (pers. commun. of the farmer).

In the olive grove, of the pesticides applied in 2018 and 2019 (Table S1), three were detected in the soil, with difenoconazole at the highest average concentration (Table 1). Of applied insecticides, such as, dimethoate, fenoxycarb and thiamethoxam, and herbicide glyphosate, their residues were not detected in the soil samples.

Physical and chemical soil properties are presented in Table 1, with the organic matter content and water capacity of the loamy soil in the vineyard higher than that of the red-brown soil in the olive grove. Soil pH was neutral to alkaline (Table 1).

In carabids, a total of nine substances were detected: six in the samples from the vineyard (VP) and five in samples from the olive grove (OP), (Tables 1 and 2). Of the detected pesticides, valiphenalate was at the highest concentration in carabids collected in the vineyard and azoxystrobin in those collected in the olive grove (Table 1). Difenoconazole was detected in carabids from both sites, whereas the insecticides dimethoate and fenoxycarb were detected in carabids but not in the soil in the olive grove. Of the insecticides, thiamethoxam was only applied in 2018 and was not detected in the carabids collected in 2019, nor in the soil samples. Pesticides detected in carabids and in the soil are presented in boxplots in Fig. 2.

Bioaccumulation factors (BAF) based on pesticide tissue concentrations relative to environmental pesticide concentrations revealed that the amount of azoxystrobin (BAF = 5.12 in OP), valiphenalate (BAF = 2.09 in VP), metalaxyl-M (BAF = 1.41 in VP) and difenoconazole (BAF = 1.16 in VP and 1.24 in OP) were higher in the carabids than in the soil, which indicates bioaccumulation.

Three active ingredients, two insecticides and one fungicide, were detected in the carabids but not in the soil (Fig. 3).

DISCUSSION

Here the amount of pesticide in carabid beetles and the soil in a vineyard and olive grove, and bioaccumulation of these pesticides in carabids is recorded. In addition, the relationship between treatment intensity of pesticides and level at which they can be detected is discussed.

Fungicides detected in carabids

In the vineyard, 18 fungicides were applied in the two years (Table S1) in order to control Phomopsis cane and leaf spot, (*Phomopsis viticola*), downy mildew (*Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni, (1888)), powdery mildew (*Uncinula necator* (Schwein.) Burrill, 1892) and grey mould (*Botrytis cinerea* Persoon, 1794) infesting grapes, which are the most economically significant and frequent diseases at the site studied (pers. commun. of farmer).

Of the applied fungicides, azoxystrobin, difenoconazole, dimethomorph, metalaxyl-M, pyriofenon, spiroxamine,

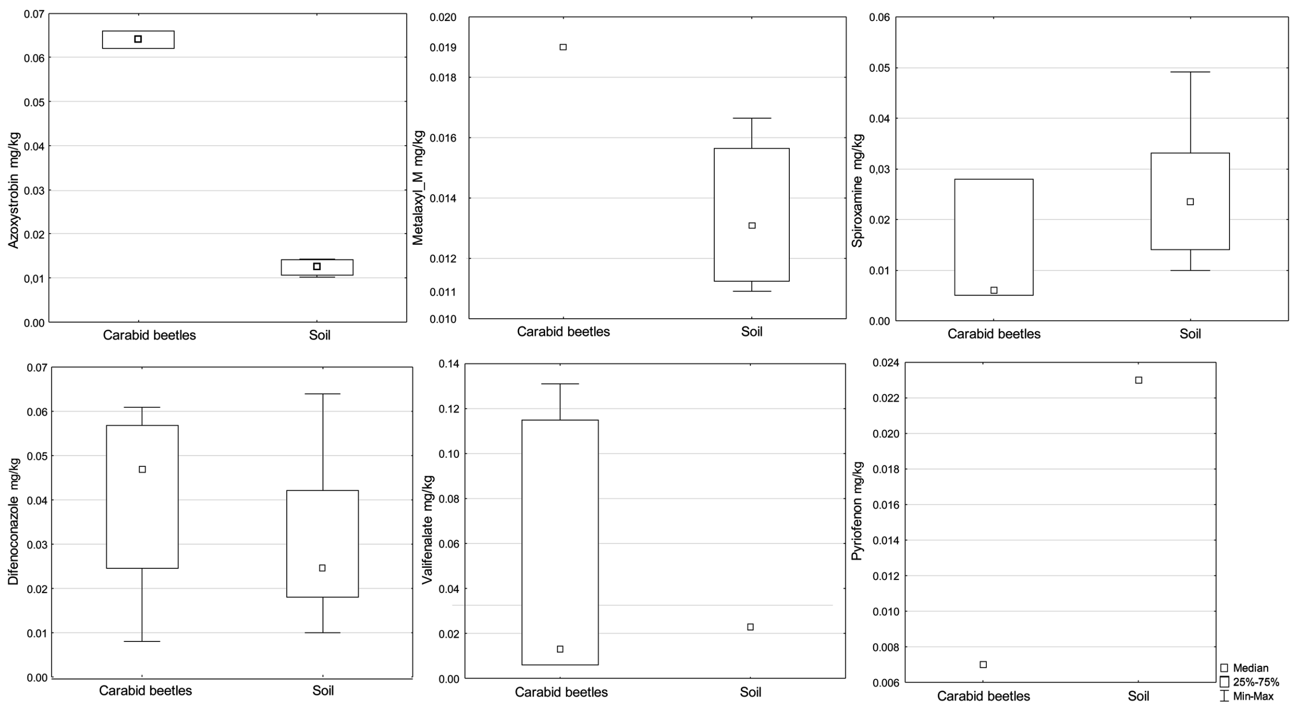

Fig. 2. Box plots showing median, minimum, maximum and quartiles of detected amounts of pesticides in carabids and soil samples.

valifenalate were detected in carabids probably because they consumed contaminaded material or via contamination of the surface of their bodies (Table 1). Their application usually occurs after the first mulching of the grass, which coincides with the breeding of the carabids, which makes it likely that they were directly treated with these active ingredients.

Azoksystrobin is a broad-spectrum QoL fungicide. Based on the assessment of EFSA (2010), the in-field and off-field risk for non-target arthropods is low. It may persist in soil and in water ecosystems under certain environmental conditions. Geisen et al. (2021) report a higher concentration of azoxystrobin in the soil under vegetables than in the soil in orange orchards and vineyards. The timing of the application of azoxystrobin in the olive grove was determined by the need in spring to protect olive from infestations of *Spilocaea oleaginea,* which is when carabids become active (Table 1 and 2), and the bioaccumulation of the active ingredient was reported in carabids (Figs 2 and 3). Difenoconazole can be applied as a seed treatment, foliar spray and systemic fungicide. According to EFSA (2023a), the risk to bees and non-target arthropods is low. The risk of it becoming chronic is reported for earthworms due the active ingredient and its metabolite CGA 205375 (EFSA, 2023a). Here, difenoconazole was used as a foliar spray, in 2018, at both sites, and was detected in soil and carabids at both sites, even a year after application, which indicates it persists in ecosystems (Silva et al., 2019). Although Silva et al. (2019) did not list difenoconazole among the most abundant active ingredients in European soils, it is persistent and therefore in future studies it should be considered.

The index of treatment with difenoconazole for the sites studied was high (Table S1) because it is legal to frequently apply this pesticide, especially in olive groves. The time of application also coincides in olive groves with the activity of both spring and autumn carabid breeders and the period for protecting olives from peacock's eye. Bioaccumulation in the carabids, *Pterostichinae* and *Harpalinae*, was detected, and in *Pterostichinae* at both sites (Fig. 3, Table 2). Possible transmission of difenoconazole in the food consumed should also be considered, and it is suggested that in future studies not only adult carabids but also their larvae are studied. Since it is applied in spring and autumn, a possible negative effect on the developmental stages should be considered in future studies (Ding et al., 2024; Qiu & Chen, 2024). Ivanković Tatalović et al. (2020) report asymmetric body shape in populations of *Pterostichus melas* (Creutzer, 1799) at the same sites. Fluctuating asymmetry of shape of the body is used in the assessment of stress levels that affect organisms during their development, known also as developmental instability.

Dimethomorph is a fungicide effective against Oomycetes, especially Peronosporaceae and Phytophthora spp. (but not *Pythium* spp.) infesting vines, potatoes and tomatoes, but not olives (National Centre for Biotechnology Information, 2024a). According to Silva et al. (2019), in European soils it is present on average one percent more than difenoconazole and is moderately persistent according to Geissen et al. (2021). Dimethomorph was applied in the vineyard (Table S1), and was detected in carabids at both sites (Table 2). According to EFSA's (2023b) pesticide risk based on the available data and risk assessment, in-field and off-field, is classified as low, including for *Poecilus cupreus* (Linnaeus, 1758). Dimethomorph and difenoconazole were detected in carabids at both sites (Table 1 and 2). The fact that it was recorded in the olive grove, where

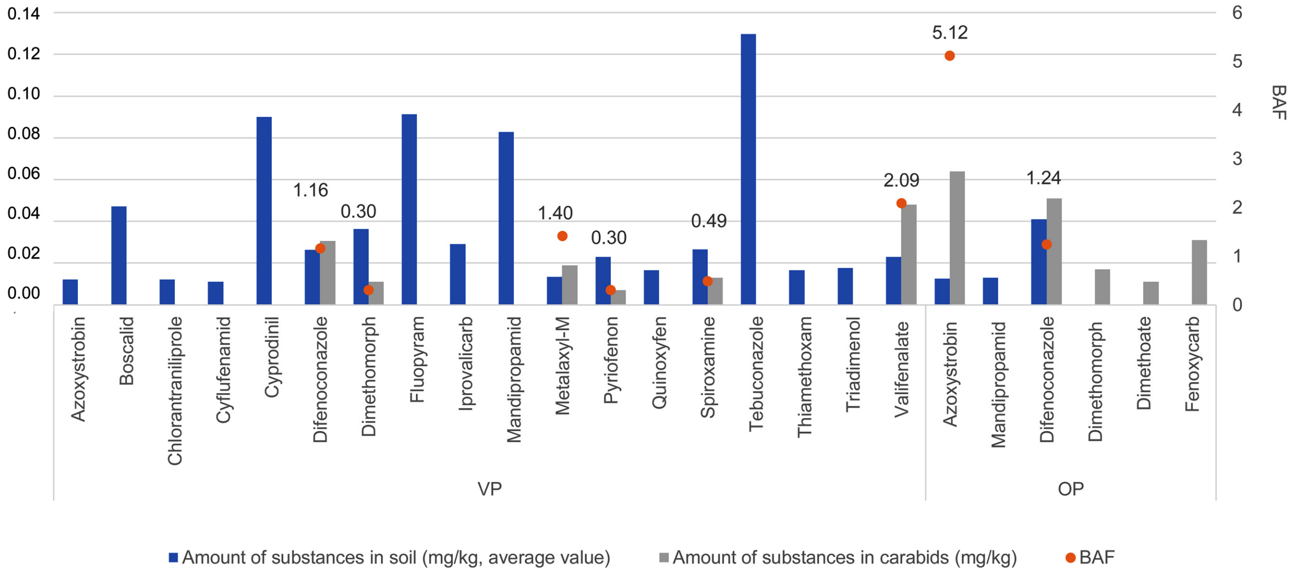

Fig. 3. Amount of active ingredients in the soil (two-year average) and in carabid beetles (mg/kg dry weight), and the bioaccumulation factor (BAF – values on secondary axes) for carabids in the IPM managed vineyard (VP) and olive grove (OP).

it was not applied, is worrying. A general problem at the level of the average Mediterranean agricultural landscape in Croatia is that the fields are small, up to 1 ha, or less. The probability of movement of carabid beetles is relatively high (e.g. Russon & Woltz, 2014) so they may come into contact with pesticides elsewhere. Carabids may move back and forth between various crops and colonize agricultural fields when the availability of food there is high. They move to other habitats, when crops become unsuitable for carabids, especially when stone fruit, viticulture, olives and vegetables are sprayed with fungicides (Russon & Woltz, 2014).

Metalaxyl-M is a systemic fungicide, which according to EFSA (2023c), pose no risks for non-target arthropods. Silva et al. (2019) do not list it among the more common fungicides in European soil, but according to Geissen et al. (2021) it is moderately persistent and often accumulates in the soil in vineyards. The measurements presented are similar to those in the literature with the addition that it is bioaccumulated by ground beetles (Fig. 3), for example in the predatory carabids *Pterostichus melas* and *Poecilus koyi*, even though the treatment index was low (Table S1); however, the time of application is from end of May to the middle of July, which coincide with the most intensive mulching of the grass and the presence of spring active carabids on the surface of the ground.

Pyriofenone can be used as a foliar fungicidal spray, for controlling powdery mildew infesting grapes. Its mode of action is not fully understood as it causes the collapse of cells in the fungus. In Croatia it was used from 2019, when it was legally permitted. The risk was assessed as low for honeybees, non-target arthropods and earthworms (EFSA, 2013a). Accumulation in soil and ground beetles is not currently reported in the literature, although metraphenone, a molecule similar to pyriofenone, frequently persists in soil under vegetables (Geissen et al., 2021). This substance, however, was sprayed twice in 2019 in the middle to the end of May, and there is a possibility that the *Pseudophonus rufipes* collected in July came into contact with this pesticide by ingesting it in their food. Since its application intensity was low, only 75g/ha, it could pose a significant threat to non-target fauna and be transmitted by organisms, and therefore it is suggested that additional studies on its bioaccumulation should be carried out in the future.

Spiroxamine is a systemic fungicide used to control common fungal diseases of cereals and fruit, and powdery mildew in grapes. It poses a high risk for fish (EFSA, 2021) and birds (Lewis et al., 2016), moderate risk for honeybees, but there is no information on its effect on non-target arthropods and ground beetles (EFSA, 2021). In the current study it was detected (Table 1 and 2) in *Poecilus koyi* and some Harpalinae sampled in July (Fig. 2), but the application dose per unit area was low, 250g/ha (Table S1).

Valifenalate is a carboxylic acid amide and an anti-peronosporic fungicide, used to control mildew in grapes and many other crops (National Centre for Biotechnology Information, 2024b). The risk posed by this fungicide is assessed as low for bees, non-target arthropods, earthworms and soil microorganisms (EFSA, 2013b). For carabids, there is the same risk of possible bioaccumulation in vineyards, as is the case for pyriofenone, because the spring applications of these three substances overlap or are separated by no more than a month. Valifenalate is not persistent in soil and is soluble in most organic solvents and is prone to bioaccumulation (Lushchak et al., 2018), which was corroborated by the data presented. In addition, valifenalate was detected in carabids at higher concentrations than other active ingredients (Table 1). This is worrying given the application dose of valifenalate per unit area was very low, 120g/ha. Concentrations of valifenalate in carabids ranged from 0.006 to 0.115 mg/kg, indicating even higher bioaccumulation potential, but these differences may also

Table 2. Samples of carabid beetles were pooled and the amount of active substances detected in the samples collected at the sites recorded: vineyard (VP) and olive grove (OP).

Site	Pool	Month 2019	Mass (g)	N ind.	Species with number of pooled specimens	Amount of substances mg/kg								
						Azoxystrobin	Difenoconazole	Dimethoate	Dimethomorph	Metalaxyl-M	Fenoxycarb	Pyriofenon	Spiroxamine	Valifenalate
VP	1	July	0.998	8	*Pseudophonus rufipes*									0.115
	2	July	1.060	8	*Pseudophonus rufipes*								0.006	0.131
	3	July	1.004	9	*Pseudophonus rufipes*									0.006
	4	July	1.010	7	*Pseudophonus rufipes*							0.007		0.006
	5	July	0.880	7	*Poecilus koyi*		0.008							0.007
	6	July	0.710	6	*Poecilus koyi*								0.005	0.013
	7	July	0.650	7	*Harpalinae*		0.053						0.028	0.058
	8	June	1.098	1	*Carabus coriaceus*									
	9	June	0.608	1	*Carabus coriaceus*									
	10	May	0.800	6	*Pterostichus melas* 2 ind. *Poecilus koyi* 4 ind.					0.019				
	11	May	1.010	19	*Harpalinae*									
	12	Oct.	0.821	5	*Pterostichus melas*				0.011					
OP	1	July	1.000	7	*Pterostichus melas* 2 ind. *Poecilus koyi* 3 ind. *Harpalus* spp. 2 ind.			0.011						
	2	June	1.090	5	*Pterostichus melas*	0.062	0.061				0.024			
	3	June	1.088	6	*Pterostichus melas* 5 ind. *Poecilus koyi* 1 ind.	0.066	0.041				0.038			
	4	May	1.316	1	*Carabus coriaceus*									
	5	Sept.	1	3	*Pterostichus melanarius*				0.017					
	6	Sept.	1.104	1	*Carabus coriaceus*									
N pools	18		N ind.	108										

be a consequence of their mobility and the different periods carabids were exposed to pesticides at the sites studied.

Insecticides detected in carabids

The use of zoocides compared to fungicides was significantly lower, with three used in 2018 and one in 2019 (Table S1). Zoocides were used to control the American grapevine leafhopper (*Scaphoideus titanus* Ball, 1932), the carrier of Grapevine flavescence dorée phytoplasma, and the vine mealybug (*Planococcus ficus* Ben-Dov, 1994) and European red mite (*Panonychus ulmi* (Koch, 1836)).

Of the insecticides applied, dimethoate and fenoxycarb were detected in carabids, but not the neonicotinoids, in particular thiamethoxan and clothianidin, which was expected as neonicotinoids were banned by EU in 2018 (EU Commission, 2018). After their ban, these insecticides were not applied in 2019, when carabids were sampled. Dimethoate and fenoxycarb are organophosphate and carbamate insecticides, and regardless of the fact that they were not detected in the soil (Table 1), their transfer through ecosystem and presence in carabids was confirmed (Table 2).

Dimethoate is a synthetic organic thiophosphate with a camphor like odour and exposure to it is by contact, ingestion and inhalation (Mauchline et al., 2004; National Centre for Biotechnology Information, 2024a). There does not appear to be any publications on its bioaccumulation in ground beetles, although EFSA (2018) states that the risk for non-target arthropods is high.

Fenoxycarb is a carbamate ester that is a juvenile hormone mimic (National Centre for Biotechnology Information, 2024b). This zoocide in soil is immobile and dissipates rapidly. Primary and secondary half-lives range from 3.07 to 5.11 days and 13.7 to 44.9 days, respectively (McDonald, 1995 in Sullivan, 2000). This indicates that unless ground beetles are treated directly, the probability of bioaccumulation is very low, as was also corroborated by the results of this study.

Exposure to insecticides like thiamethoxam (Ivanković Tatalović et al., 2023) can have negative sub-lethal effects in the activity of predators and their energy budget. Low concentrations of neonicotinoids are stimulatory, whereas high concentrations result in paralysis and death (Goulson, 2013). Sublethal effects can negatively affect the efficiency of predators and herbivores in pest and weed control (e.g. Elzen 1990; Pisa et al., 2015; Yao et al., 2015). The specimens analysed in this study, survived the application of insecticides, but accumulated compounds they were unable to eliminate from their body, and despite low concentrations, these pesticides might harm carabids. Merivee et al. (2015) confirm that low doses of the common alpha-cypermethrin insecticide affect the thermoregulation of the carabid *Platynus assimilis* (Paykull, 1790).

Potential effect of other pesticides used at the sites studied on carabid beetles

The herbicide, glyphosate, was not detected in the soil and carabids. In both years, glyphosate was used to control weeds in the vineyard. Based on data and the risk assessment, the threat to non-target arthropods other than bees is low (EFSA, 2023d). Nevertheless, El Jaouhari et al. (2023) emphasize that the effect of herbicides is related to a cascading effect on the food web due to the removal of a primary trophic resource. So, in the future, research should focus on the effect of glyphosate on carabid abundance in terms of its effect on the availability of prey as Lewis et al., (2016) state that glyphosate adversely affects earthworms, which are a preferred prey (Šerić Jelaska et al., 2014a, b; Šerić Jelaska & Symondson, 2016) rather than its toxicity for carabids.

Among the pesticides recorded in the soil, boscalid and chlorantraniliprole should be considered in future studies on bioaccumulation. Boscalid and chlorantraniliprole persist in soil (Lewis et al., 2016; Silva et al., 2019). Boscalid is among the broad-spectrum fungicides that are most frequently reported at high concentrations in European soils (Silva et al., 2019). Its solubility in water is low and despite occasionally exceeding it predicted environmental concentrations in soil, the toxic levels are below those for soil organisms (Silva et al., 2019). Chlorantraniliprole is an insecticide recently used against lepidopteran pests. Both of these pesticides were not used in the vineyard during this study, but were used a year before this study and were recorded in the soil in the vineyard at high concentrations, which confirms their persistence in soil and low mobility at least for carabids. Boscalid was not applied, so its presence in soil is possibly due to the drift of spray from a neighbouring apple orchard.

Bioaccumulation of detected pesticides and potential risks to carabids as non-target organisms providing ecosystem services

Of nine synthetic pesticides detected in carabids, four were accumulated in their body, with the concentrations being higher than in soil samples, and difenoconazole was accumulated in carabids collected from both plantations. The accumulated pesticides pose the risk of spreading contaminants through ecosystem via predation on carabids.

Two pesticides were detected in soil, but not in carabids. Carabids might be able to detoxify pesticides (Kramarz, 1999) or decrease food intake (Maryanski et al., 2002), but their accumulation in the bodies of carabids may indicate that their detoxication efficiency is low. On the other hand, detoxification is highly energy demanding (Calow, 1991; Ivanković Tatalović et al., 2023) and exposure to pesticides and xenobiotics in the environment can be harmful despite not being detected in their body or present at very low concentrations and even when animals survive pesticide treatment, it can impair their hunting abilities, metabolism and locomotion. Ivanković Tatalović et al. (2023) report that individuals treated in vitro with high concentrations of thiamethoxam consume significantly less food per unit body weight and many individuals become intoxicated or moribund. Furthermore, there are significant differences in concentrations of some metabolites in treated and control individuals, mainly in succinate and d-glucose, indicating a disruption of energy production (Ivanković Tatalović et al., 2023). Tooming et al. (2017) confirm the hypoactivity, reduction in food consumption and changes in the behaviour of carabids after exposure to sub-lethal doses of some insecticides.

All this indicates that despite being mobile, moving between crops and being able to detoxify pesticides, carabids are nevertheless exposed to synthetic ingredients, which they deal with metabolically or with reduced locomotion. The low-dose but continuous (long-term) exposure to pesticides can impair carabid hunting abilities or fecundity and thus negatively affect the ecosystem services they provide. Carabid ecosystem services can reduce the need for using pesticides in ecosystems like Mediterranean agricultural fields, on carbonate rock, with underground water systems that can be easily contaminated with pesticides used throughout the landscape. Encouraging predatory arthropods and increasing their role in such ecosystems might reduce the need to use pesticides.

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Supplementary Table S1 follows on next page.

Table S1. Amount of the active substances of pesticides applied per hectare at the integrated pest management sites studied in the years 2018 and 2019. A.I. – Active ingredient, I.T. – Intensity of treatment, M.N.T. – Maximum number of treatments/season. The maximum number of applications permitted per year was determined by the phytosanitary department of the Croatian Ministry of Agriculture for the current year. I.T. (%) is the percentage of A.I. in M.N.T.

Active substance	Vineyard						Olive grove					
	2018			2019			2018			2019		
	A.I. (g/ha)/y	I.T. (%)	M.N.T.	A.I. (g/ha)/y	I.T. (%)	M.N.T.	A.I. (g/ha)/y	I.T. (%)	M.N.T.	A.I. (g/ha)/y	I.T. (%)	M.N.T.
copper	2,290.00	25.00	4.00	4,220.00	26.66	4.00	4,500.00	37.50	3.00	7,500.00	55.55	3.00
sulphur	6,580.00	41.67	8.00	12,000.00	46.66	8.00	1,600.00	12.50	1.00	1,600.00	11.11	1.00
Fungicides												
azoxystrobin	–	0.00	4.00	235.00	6.67	4.00	250.00	12.50	1.00	250.00	11.11	1.00
cyazofamid	–	0.00	8.00	200.00	13.33	8.00	–	0.00	–	–	0.00	–
cyflufenamid	36.00	16.66	2.00	–	0.00	2.00	–	0.00	–	–	0.00	–
cymoxanil	–	0.00	6.00	113.00	6.67	6.00	–	0.00	–	–	0.00	–
cyprodinil	975.00	16.66	2.00	675.00	13.33	2.00	–	0.00	–	–	0.00	–
difenoconazole	50.00	16.66	4.00	72.00	13.33	4.00	383.00	50.00	4.00	588.00	44.44	4.00
dimethomorph	226.00	8.33	5.00	–	0.00	5.00	–	0.00	–	–	0.00	–
fludioxonil	500.00	16.66	2.00	200.00	6.67	2.00	–	0.00	–	–	0.00	–
fluopyram	80.00	8.33	2.00	–	0.00	2.00	–	0.00	–	–	0.00	–
folpet	2,700.00	25.00	5.00	1,920.00	26.67	5.00	–	0.00	–	–	0.00	–
fosetil	4,225.00	25.00	6.00	4,000.00	20.00	4.00	–	0.00	–	–	0.00	–
iprovalicarb	246.00	16.66	3.00	–	0.00	3.00	–	0.00	–	–	0.00	–
isofetamide	–	0.00	–	600.00	6.66	1.00	–	0.00	–	–	0.00	–
mancozeb	3,520.00	25.00	4.00	3,200.00	13.33	4.00	–	0.00	–	–	0.00	–
mandipropamid	30.00	8.33	4.00	375.00	20.00	3.00	–	0.00	–	–	0.00	–
meptyl-dinocap	210.00	8.33	4.00	–	0.00	4.00	–	0.00	–	–	0.00	–
metalaxyl-M	152.00	16.66	3.00	47.50	6.67	4.00	–	0.00	–	–	0.00	–
penconazole	25.00	8.33	4.00	30.00	6.67	4.00	–	0.00	–	–	0.00	–
pyriofenone	–	0.00	2.00	975.00	13.33	2.00	–	0.00	–	–	0.00	–
quinoxyfen	50.00	8.33	5.00	62.5	6.67	5.00	–	0.00	–	–	0.00	–
spiroxamine	125.00	8.33	2.00	500.00	13.33	5.00	–	0.00	–	–	0.00	–
tebuconazole	240.00	25.00	3.00	–	0.00	5.00	–	0.00	1.00	–	0.00	1.00
triadimenol	23.00	8.33	3.00	–	0.00	3.00	–	0.00	–	–	0.00	–
valifenalate	–	0.00	–	240.00	13.33	2.00	–	0.00	–	–	0.00	–
zoxamide	–	0.00	5.00	120.00	6.67	5.00	–	0.00	–	–	0.00	–
Zoocides												
dimethoate	–	0.00	0.00	–	0.00	–	1,200.00	25.00	2.00	2,000.00	33.33	2.00
etoxazole	–	0.00	1.00	30.00	6.67	1.00	–	0.00	2.00	–	0.00	2.00
fenoxycarb	–	0.00	2.00	–	0.00	2.00	–	0.00	–	15.00	11.11	1.00
pyriproxyfen	50.00	8.33	1.00	–	0.00	1.00	–	0.00	1.00	–	0.00	1.00
spirotetramat	100.00	8.33	2.00	–	0.00	2.00	–	0.00	1.00	–	0.00	1.00
thiamethoxam	50.00	8.33	3.00	–	0.00	–	–	0.00	–	–	0.00	–
Herbicides												
glyphosate	900.00	8.33	1.00	600.00	6.67	1.00	–	0.00	1.00	–	0.00	1.00
Total number of treatments/years		12.00			15.00			8.00			9.00	