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EFFECTS OF TWO HERBICIDES ON STRAWBERRY PLANTS

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Abstract

Herbicide substances aminopyralid and pyroxsulam are used to control broadleaf weeds in cereals. Disadvantage of this herbicides is fact, that their residues remain not only in the soil, but are also translocated to the plant tissues of the treated crops. Herbicide residues released from straw used like mulch by the way of decomposition and leaching can damage strawberry. In this study the response of strawberry plants to aminopyralid at doses of 3.75, 7.5 and 15 g/ha and pyroxsulam at dose 3, 15 and 30 g/ha was evaluated. With the increased concentration of both herbicides, plants injury also increased. A reduction in fruit yield was noted with both herbicides and cultivar 'Elsanta' was more sensitive than cultivar 'Karmen'. Mustang Forte herbicide at the highest rate reduced the yield of the 'Elsanta' cultivar by 52% and the 'Karmen' cultivar by 47% in the first year. However, in the second year, the decrease in yields of both varieties leveled off at 30% compared to the control. Similarly, in the case of pyroxsulam, the yield of cultivar 'Elsanta' decreased by 70% in the first year, while yield of cultivar 'Karmen' only decreased by 55%, but here the difference between cultivars remained until the second year.

Keywords: aminopyralid, auxin, crops injury, enzyme acetolactate synthase (ALS), flowering, *Fragaria* × *ananassa*, fruit, pyroxsulam, herbicide residues

INTRODUCTION

Strawberries (*Fragaria* × *ananassa)* are among the most popular fruits worldwide due to their great taste, aromatic smell and high content of minerals and vitamins (Edger *et al.*, 2019; Yuan and Sun, 2022). In terms of vitamin C content, strawberries take third place after rose hips (*Rosa canina)* and black currants (*Ribes nigrum)* (Giampieri *et al.*, 2013). The most important exporters of strawberries in 2021 were Spain (316 572 t), Mexico (182 540 t), the United States of America (137 501 t), Greece (136855 t) and the Netherlands (64 832 t) (World Integrated Trade Solution, 2022).

A condition for commercial strawberry cultivation is high-quality, healthy and weed-free stands. Therefore mulch straw is used around the plants to prevent the growth of weeds and keep the fruit clean (Daugaard, 2008). However, if the origin of the straw used is unknown, it may contain residues of various herbicides used in grain cultivation, which can hinder the growth of strawberry plants. These residues are released from straw used as mulch by rain and can harm sensitive plants, even in very small amounts (Fast *et al.*, 2011; Soukupova and Koudela, 2023).

Herbicides from the pyrimidine group with the active substances as aminopyralid, pyroxsulam, picloram and clopyralid are preparations based on the analogue plant hormone auxin (Wells, 2008). These modern herbicides are used to control broadleaf weeds in crops and grassland. The disadvantage of these non-toxic substances for animals and humans (Commission, 2013; EFSA, 2013) is that they decompose slowly in the soil (Gilbert *et al.*, 2010) and their residues attach to the plant tissues of the treated crops (Ferrell *et al.*, 2020) having a problematic effect when subsequently growing sensitive crops, especially from the Fabaceae and Solanaceae families (Fast, 2010).

Aminopyralid (chemical name 4-amino-3,6-dichloropyridine-2-carboxylic acid) is a selective, systemic post-emergence and pre-emergence herbicide (Dow AgroSciences, 2015), that is effective on many persistent and invasive broadleaf weeds (McManamen, Nelson and Wagner, 2018). It is mainly used to treat cereals, pastures and grass. Aminopyralid is a synthetic auxin herbicide of the pyridinecarboxylic acid family. The active substance aminopyralid is not used alone. Most often, it used in combination with the substances clopyralid and picloram (commercial herbicides, e.g. Blast, Bonaxa, Galera) or in combination with florasulam (herbicides, e.g. Huricane, Kantor, Mustang Forte) or with other substances as a third component such 2 ,4-D, metazachlor (Metazamix) or pyroxsulam (Huricane).

Auxin herbicides are rapidly absorbed by the plant and distributed through the phloem to tissues with growth activity, resulting in various growth and reproductive anomalies (Jursík, Holec and Soukup, 2009; Ferrell *et al.*, 2020) Auxins are involved in many processes during fruit development; these levels are usually high early in development and decline at or before the onset of ripening, suggesting a possible role in ripening inhibition (Wu *et al.*, 2018). In some fruits, auxin treatment delayed ripening (Yuan *et al.*, 2019; Li *et al.*, 2022), the specific effects of ripening-delaying auxin treatments include delayed reduction of chlorophyll levels, delayed changes to cell wall components (fruit softening), and delayed accumulation of sugars (or other forms of carbon storage) (Davies and Böttcher, 2014). Studies performed on strawberry (Villarreal, Martínez and Civello, 2009; Symons *et al.*, 2012; Fan *et al.*, 2022) have suggested that auxin can slow down the ripening of this non-climacteric fruit.

Pyroxsulam (chemical name N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfon amide) is a post-emergence herbicide, has both foliar and soil activity, and is translocated to growing points where it inhibits the enzyme acetolactate synthase (ALS). Blocking the function of ALS has the immediate effect of stopping the production of amino acids and, consequently, proteins, thus stopping the growth of the plant (Soukup and Holec, 2010). Its advantages include that it gives control of annual grass and broadleaf weeds, is applied at a low rate of active ingredient per hectare, is selective to wheat, and it is environmentally friendly and non-toxic to most organisms, including humans (Wells, 2008).

The active substance pyroxsulam is used alone in the herbicides e.g. Corello, PowerFlex, Crusader, or in combination with the herbicide substance florasulam (herbicides, e.g. Huricane, Ataman, Orcane, Prodigy), or other substances (e.g. halauxifen-methyl in Orcane) as a third component.

ALS is one of the key enzymes in the biosynthesis of essential amino acids (valine, leucine, isoleucine). Blocking the function of ALS stops the formation of these amino acids and proteins (Soukup and Holec, 2010). Despite the fact that ALS inhibitors have been used as herbicides for more than 40 years and more than 50 active substances are registered (Widianto *et al.*, 2022), very few studies have been conducted on their effect on subsequently cultivated plants.

The manufacturers of these herbicidal substances in the leaflets of the herbicides state a warning against the use of straw from treated grain as mulch for strawberry or mushrooms (Dow AgroSciences, 2008; Corteva, 2019). Currently, however, only a few studies have dealt with the effect of the residues of these herbicides on vegetables or fruit, but none of them have dealt with strawberries. Therefore, the aim of this study was to evaluate the effect of aminopyralid and pyroxsulam in two forms, as a pure substance and as a commercial herbicide, on strawberry plants on their damage, fertility and fruit yield. It was also to determine a critical level of herbicide concentration for strawberry plant damage and to investigate if there was any difference in tolerance to aminopyralid or pyroxsulam damage between the two cultivars.

MATERIALS AND METHODS

Two cultivars 'Karmen' ('K') and 'Elsanta' ('E') frigo seedlings were bought from farm Hanč Vraňany (Czech Republic). Bought cultivars are old varieties commonly used in horticultural practice (Sieczko *et al.*, 2015; Sowik, Markiewicz and Michalczuk, 2015).

The substrate "Agroprofi Jahody 1676" (AGRO CS a.s., Česká Skalice, CZ) was used for plant cultivation. Compositions: white peat (60%), black peat (20%), clay (40 kg/m), osmocote extract (2 kg/m), coconut fibre (20%) and finely granulated Micromax fertiliser (0.15 kg/m).

The studies with plants were conducted in 2022 and 2023 at the Demonstration and Research Station (Czech Republic) and took place from March 2022 to July 2023.

Strawberry seedlings with the five true leaves, 15 phase according to BBCH (Meier, 2001) identification, were planted in experimental pots volume 5 L one month before herbicide application. Pots with strawberry plants were localised in a foil tunnel open from the south and north sides with dimensions of 14 × 9 m. Drip irrigation was introduced into individual plant pots, and the plants were watered daily 0.5 L per plant.

Both herbicides and pure substances were applied once to the soil 1 month after planting (1.039 mL in 100 mL of distilled water). This dose was calculated based on the determination of dry matter in the substrate (43%), the volume of the pots (5 L) and the optimal spray rate (300 L/ha) according to Jursík *et al.* (2009). The same volume of distilled water was used for the control group. The experimental plants, which were planted in the spring of 2022, were treated once with herbicides and observed during the coming period until 2023.

Aminopyralid (AM) purity 96% (Shanghai Tianfu Chemical Ltd, Hong Kong, China) and herbicide Mustang Forte (MF) (Dow Chemical Company, Midland, USA), active substances 2,4-D (180 g/L), AM (10 g/L), and florasulam (5 g/L), was applied at corresponding doses: C1 = 3.75, C2 = 7.5 and C3 = 15 g/ha of AM. We relied primarily on similar studies (Fast *et al*., 2011; Soukupova and Koudela, 2023).

Pyroxsulam (PY) purity 95% (Shanghai Tianfu Chemical Ltd, Hong Kong China) and herbicide Corello (CO) (Dow Chemical Company, Midland, USA), active substance PY (75 g/kg), were applied at corresponding doses: C1 = 3, C2 = 15 and C3 = 30 g/ha of PY. Was relied on the methodology similar studies (Singh *et al*., 2010; Chhokar, 2019).

The herbicides were applied evenly on the soil surface without contact with the above ground part of plants. Repetition was 10 plants for each concentration. All plants were in the stage before the formation of offshoots and flowers, before the 41 phase according to BBCH identification.

After herbicide application, plants were monitored twice a week. In each term, the following parameters were evaluated: plant injury in % evaluated as twisting of leaves, damage (drying) to flowers, chlorosis and overall condition of the plant. The scale of injury is provided in supplementary material Fig. S1. The number of ripe fruits and their weight (given as total plant yield in g in one harvesting) were determined. The evaluation was based on the modified methodology of studies with clopyralid on strawberry plants (Hunnicutt *et al*., 2013; Sharpe *et al*., 2018).

The measured values were evaluated by analysis of variance (ANOVA) and Fisher's least significant difference (LSD) method using TIBCO Statistica Ultimate Academic software (version 13.5; StatSoft (Europe) GmbH, Hamburg, Germany). For all analysis was used ANOVA. The purpose of analysis of variance (ANOVA) is to test for significant differences between means by comparing (i.e., analyzing) variances. Unweighted means was used because if the cell frequencies in a multifactor ANOVA design are unequal, the unweighted means (for levels of a factor) are calculated from the means of sub-groups without weighting, that is, without adjusting for the differences between the subgroup frequencies (Academic, no date) and in the case of this study, it was more appropriate to use unweighted averages, since in the first year not all plants from the 10 replicates fruited as can be seen supplementary material Tab. S1.

RESULTS

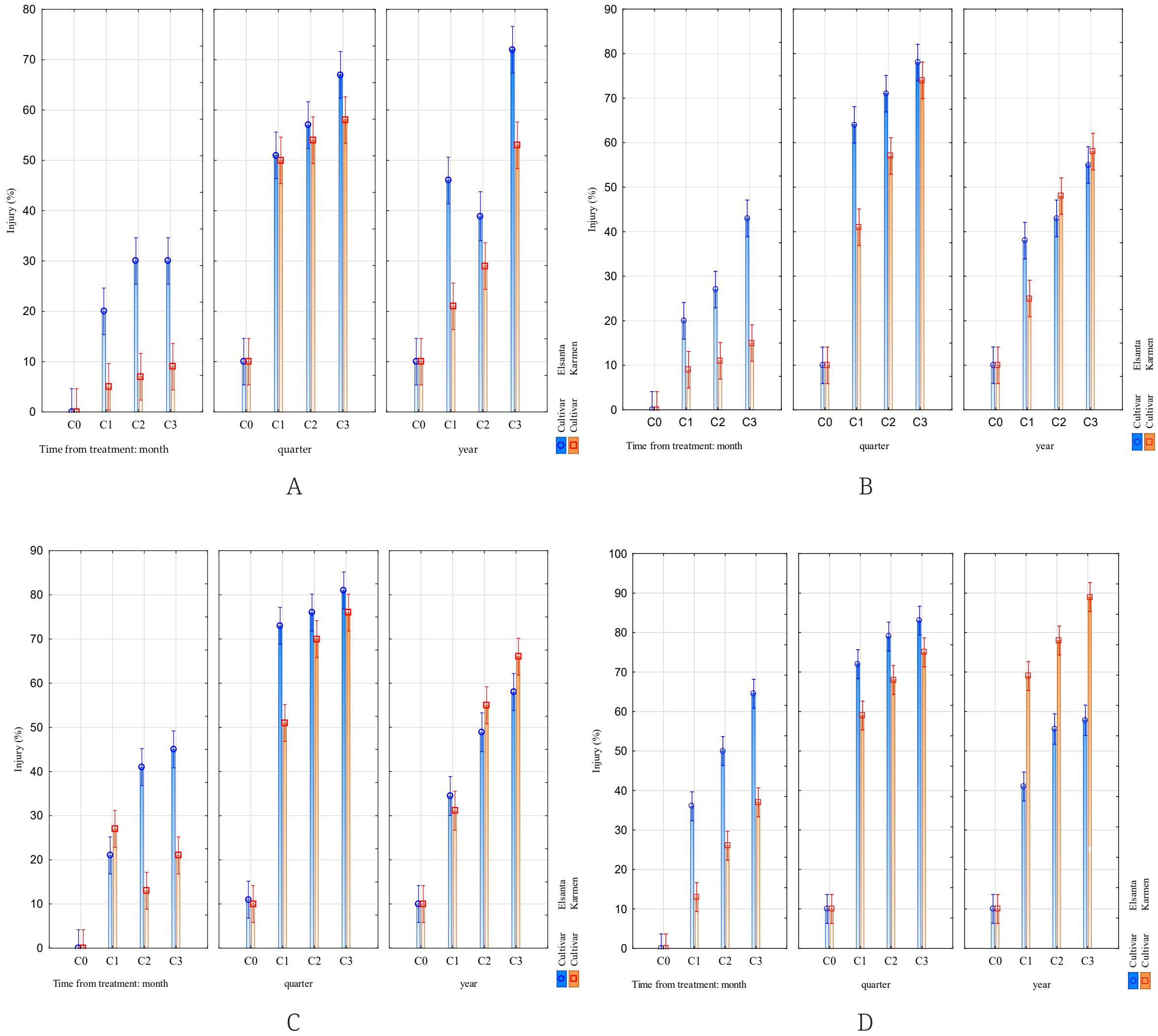
Effect of Strawberry Plant Injury

Herbicide injury to the plants was evaluated continuously throughout the experiment. Fig. 1 showed that herbicides AM and PY, in very low doses significantly injury strawberry plants. Visual symptoms were observed: deformations of the leaves, twisting of the stems and leaves, and loss of chlorosis (leaf lightening). There were deformations of the leaves, twisting of the stems and leaves, and a loss of chlorophyll. For this visual evaluation, a damage scale in percent is given in the Appendix A (Tab. A1). The condition of the plants and their injuries were evaluated in one month, after a quarter of a year and one year after treatment with the herbicide.

In the case of AM treatment, there was a statistically significant difference between the untreated control variant and all herbicide treatment concentrations, both in the case of using a pure substance (Fig. 1A) and in the case of a commercial herbicide (Fig. 1B). The choice of cultivar influenced the severity of the damage. In the case of the cultivar 'K', injury to the plants became more pronounced after only 3 months of treatment, both when using pure AM and when treated with the MF herbicide. In the cultivar 'E', signs of herbicide injury appeared to a greater extent after 1 month of treatment, and after 3 months, plant damage reached 80% when MF was used at a dose of 15 g/ha. The same dose of AM in the form of the commercial herbicide MF had a greater effect on the condition of the strawberry plants than using only the pure substance, which may be due to the other components of this preparation. In the case of PY treatment, there was a statistically significant difference between the untreated control variant and all herbicide treatment concentrations, both in the case of using a pure substance (Fig. 1C) and in the case of a commercial herbicide (Fig. 1D). The choice of cultivar did not have such an effect on damage severity as with AM, but the cultivar 'E' was injured more. However, the unexpected result was that in the case of the 'E' cultivar, the health of the plants improved more than that of the 'K' cultivar one year after treatment.

Effect on Strawberry Plant Yield

Another finding of this study was that treatment with the herbicides AM and PY led to a decrease in strawberry yield. As shown Tab. S1 in the supplementary material, not all plants from each group of 10 in the different treatments produced fruit. In first year some plants did not produce flowers at all; others had flowers or even small green fruits, but they did not reach full maturity; many of them had dried up or fallen off. In this case, a difference between the cultivars was noted. In the following year after treatment, however, all plants under observation, i.e. treated and untreated, produced fruit, but there were large differences in the number of fruits, ranging from 3 to 65 fruits per plant per season. Precisely because the strawberry had a lower yield in the first year even in the control

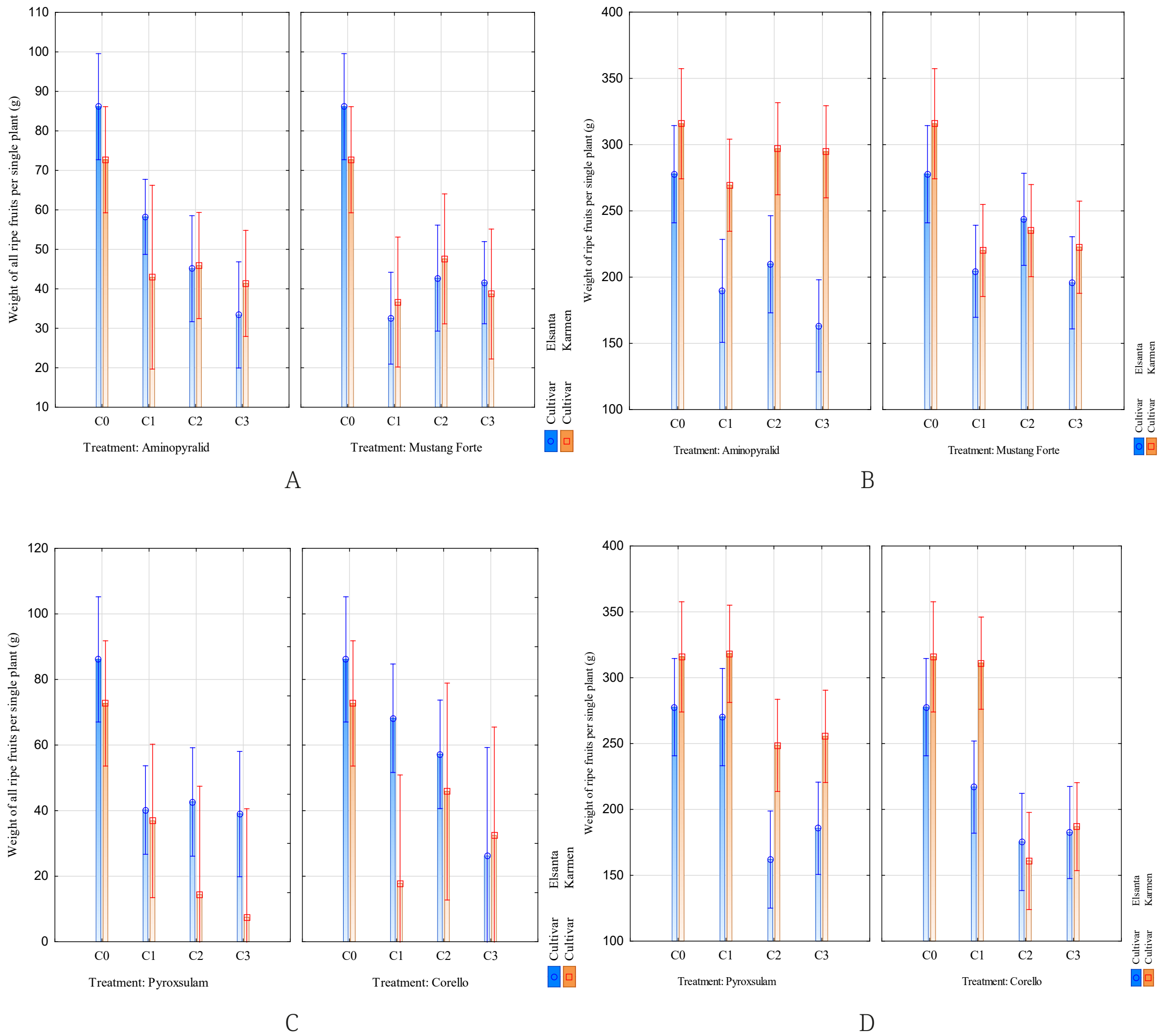


1: *Strawberry plants injury of two cultivars month, quarter and year after soil treatment with herbicide aminopyralid (A), Mustang Forte (B), pyroxsulam (C) and Corello (D), significantly different classes of values are indicated in the form of letter a, b, c*

groups, the experiment was designed for two years, so that the data from the time when the plants will already reach a market yield could be compared.

Fig. 2A shows the slight retarding effect of AM on the yield of ripe fruits per plant in grams on a single plant in the first season after treatment. This influence was again more pronounced in the cultivar 'E', it was statistically significant compared to the untreated control plants. The difference in the concentrations used was only slight; thus, it cannot be concluded that the amount of herbicide used had an effect on strawberry yield. However as shown in Fig. 2B, one year after the treatment, there was a significant increase in yield for all plants, and not only the choice of cultivar but also the form in which AM was used had a significant effect on this. This can be explained not only by the fact that MF herbicide contains other active substances, but also by the fact that it contains wetting agents that help the herbicide to penetrate faster into the plant.

Similarly, the 'E' cultivar, with the increasing concentration of PY (Fig. 2C) in both forms of use, the weight of ripe fruits was statistically significant and lower than in the control. In the cultivar 'K', the retardation of the weight of fruits was again lower, but there was no longer a clear effect of the concentration used. A statistically significant reduction in the weight of ripe fruits in this cultivar occurred in the treatment of the pure substance PY in the first season. In the year after herbicide treatment (Fig. 2D), in the case of treatment with the lowest dose of 3 g/ha in both forms, the weight of fruits per plant equalled the control group, but at higher doses, the degradation remained significant in both cultivars. The lowest concentration PY therefore has no effect in a multiyear horizon, while doses from 15 g/ha have a stronger residual effect.



2: *Effect of herbicides on the weight of ripe fruits of strawberry after soil treatment with aminopyralid during the period 2022 (A), 2023 (B) and with pyroxsulam in 2022 (C) and in 2023 (D), significantly different classes of values are indicated in the form of letter a, b, A, B*

DISCUSSION

Our data showed that both evaluated herbicides, aminopyralid and pyroxsulam, in very low doses significantly damaged strawberry plants. Visual symptoms were observed: deformations of the leaves, twisting of the stems and leaves, and loss of chlorosis (leaf lightening). These herbicides overcome the natural regulatory mechanisms of susceptible plants to cause an uncontrolled auxin response, and they cause various growth abnormalities in sensitive dicotyledonous plants (Kelley and Riechers, 2007). These deformations include curling of leaves (Ferrell *et al.*, 2020), twisting and thickening of stems (Sterling, 1997), chlorosis, and necrosis (Kelley *et al.*, 2005). A study with tobacco (Takubo *et al.*, 2020) even reported that exogenous auxin induced disorganized growth of teratoma tissue, suggesting that auxin plays a role in regulating tumor morphology. As can be seen in the pictures in Appendix A, in our study, with increasing concentration of all substances used, symptoms ranging from mild twisting of the leaves to necrosis were recorded, but we did not observe the formation of tumors in strawberry plants.

This injury of both strawberry cultivars was 60% at the lowest dose of aminopyralid (3.75 $g.ha^{-1}$) and pyroxsulam (3 $g.ha^{-1}$) compared to the untreated control plants (0%). These doses were chosen to simulate residual amounts in the soil during crop rotations, similar to Fast *et al.* (Fast *et al.*, 2011). In this study, crop injury was 48% in bell pepper, 67% in eggplant, 71% in tomato, 3% in muskmelon and 3% in watermelon compared to untreated control plants (0%). In a similar study (Seefeldt *et al.*, 2013) found that, when aminopyralid was applied at a dose of 8 g/ha, potatoes were damaged by 68% compared to the control. In this study, they also carried out an experiment with potato daughter tubers, and, similar to ours, they found that the residual effect of the herbicide was still active the year after treatment. However, we also noted a moderate ability to regenerate strawberry plants the following year, which corresponds to a study (Harrington, Peter and Devine, 2014) that showed that the frequency of wild strawberries (*Fragaria vir-giniana*) decreased after aminopyralid treatment, but the plants were able to regenerate.

Study with several types of crops (Jeffries, Mahoney and Gannon, 2014) conducted with 5 different herbicides, including aminopyralid at a dose of 12 g.ha^{-1}, and stated that this damage was 79% in cotton, 93% in pepper, 24% in squash, 70% in tobacco, and 100% in soybean and tomato. From our results and from the studies presented here, it can be concluded that most subsequently grown crops, such as vegetables from the Solanaceae family or strawberries, are sensitive to the presence of aminopyralid residues in the soil, even at very low doses of a few grams per hectare.

Another finding of this study was that treatment with the herbicides aminopyralid and pyroxsulam, either in the form of a pure substance or in a commercial preparation, led to a decrease in strawberry yield.

The manufacturers of these herbicide substances give a warning against the use of straw from treated grain as mulch for strawberry or mushrooms, but it is not stated here on what basis; they came to this warning, if any tests were carried out, or if it is just precautionary information.

Currently, however, only a few studies have dealt with the effect of the residues of these herbicides on vegetables or fruit, but none of them dealt with strawberries. In the currently available literature, we have not found any studies dealing with damage to subsequent crops using the herbicide pyroxsulam, but there are several studies looking at damage to strawberry by the herbicide clopyralid residues (Hunnicutt *et al.*, 2013; Sharpe *et al.*, 2018). Clopyralid is an auxin-type herbicide, and it is more selective than some other auxin herbicides, such as picloram, triclopyr or 2,4-D (Vasic *et al.*, 2022). First study (Hunnicutt *et al.*, 2013) evaluated three different strawberry cultivars, namely 'Treasure', 'Festival' and 'Camino Real', for sensitivity to clopyralid at spray application doses of 0, 45, 66, 132, 195 and 814 g.ha^{-1}. After weeks, the dose of clopyralid had an effect on leaf malformations in 'Treasure' and 'Festival' cultivars, but this effect was not recorded in the 'Camino Real' cultivar. Likewise, in our study with two strawberry cultivars, we also found that there was a different varietal sensitivity to residues of the herbicides aminopyralid and pyroxsulam. In a second study with clopyralid (Sharpe *et al.*, 2018) at doses of 0, 35, 70, 140, 280, 560, 1120 and 2240 g.ha^{-1}, only the strawberry cultivar 'Festival' was used. In this study, no clopyralid dose affected crop damage or plant height but did affect the leaf number. However, this statement contradicts both our study and the above-cited study (Hunnicutt *et al.*, 2013) with the same strawberry cultivar 'Festival'.

Cultivar sensitivity to herbicide treatment is also reported by studies, e.g. (Castro *et al.*, 2019; Al-Latif, 2022). On the contrary, more and more reports (Widianto *et al.*, 2022; Papapanagiotou *et al.*, 2023; Sparangis *et al.*, 2023; Zhu *et al.*, 2023) have indicated the emergence of resistance to pyroxsulam in many common, previously sensitive weeds. It is therefore a question whether this resistance to ALS blockers will gradually appear in some strawberry varieties or whether this knowledge can be used in the case of breeding new, more resistant cultivars.

CONCLUSION

The study presents results of the evaluation the effect of two herbicides based on aminopyralid and pyroxsulam on the strawberry plants in the point of view of plant injury and fruits yield reduction. The results indicates that there is significant cultivar sensitivity to auxin herbicide residues. The critical herbicides levels for strawberry plants taking into consideration cultivar specifity was determined.

With the increased concentration of the herbicides AM and PY, injury to strawberry plants also increased. Damage to fruit yield was noted for both herbicides at the lowest doses investigated. It has also been demonstrated that damage to strawberry plants by auxin herbicides persists into the next year when there is only a slight improvement in plant conditions. A longer residual effect was noted at higher concentrations of the herbicide used.

Our data also demonstrate that the cultivar 'E' was more sensitive to the effects of both herbicides, even at very low doses, than the cultivar 'K'. This cultivars sensitivity should be subjected to further study with more cultivars. The list of cultivars sensitive and resistant to these herbicides could then serve in horticultural practice as a tool for growers with selection of proper cultivar for the growing system with use of straw from cereals treated with these substances as mulch in strawberry growths, where is the potential risk of those herbicide damage.

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