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Translocation of Aminopyralid from Straw Mulch to Plants in Perennial Strawberry Plantations: Case Study

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Abstract: Aminopyralid (AP)-contaminated straw mulch is frequently used in strawberry production to maintain moisture and reduce weed growth. AP may be translocated by rain and irrigation. Contamination of plant tissues with AP during the production cycle at a strawberry farm was measured by HPLC MS/MS using a newly validated extraction method. Samples were removed from a commercial plantation using straw mulch. The highest AP levels (1.2–1.3 ng.g^{-1}) were found in strawberries; the levels in leaves and roots were two and four to ten times lower, respectively. The amounts detected in fruits were 10 times lower than the dietary tolerances given by the U.S. Environmental Protection Agency for wheat grain and cattle milk/meat. The effect of AP on flowering and fruiting was investigated in pot experiments closely mimicking farm conditions. The released AP negatively affected flowering and reduced the total fruit weight two times. The study showed a significant release of AP from mulch straw during commercial strawberry production and documented the risk of using herbicide-contaminated straw for mulching.

Keywords: strawberry production; wheat straw; aminopyralid; herbicide leaching; herbicide accumulation; fruit contamination; crop effect

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1. Introduction

Straw mulch (SM) is an effective tool for sustainable soil management in agriculture, as it helps to maintain soil moisture by effectively reducing water evaporation [1]. It is often used in the commercial production of strawberries. By covering the ground surface, SM restricts weed growth and acts as a blanket that protects above-ground parts of the plants and fruits from soil-originated pathogens and low-temperature injury [2,3]. Typically, SM is applied 10–15 cm thick to provide an insulation layer and is subject to extraction with rain and irrigation water that results in the leaching of various phenolic compounds that are toxic to plant seed germination and growth and of herbicides, with which the wheat straw is treated [4–7].

Aminopyralid (AP) is a synthetic auxin herbicide of the pyridine carboxylic acid family used to control broadleaf weeds. It has detrimental effects on sensitive broadleaf plants, potatoes, tomatoes and legumes, resulting in twisted stems, leaves and the stunting of roots, ultimately leading to necrosis, chlorosis and plant withering. Even monocots like wheat can show symptoms of epinasty and distortion in certain situations [7,8]. Experiments with small, constructed plant communities that included *Prunella vulgaris* var. *lanceolata*, *Festuca roemeri*, *Clarkia amoena* and *Cynosurus echinatus* showed that the application of AP at 16.7–61.5 g a.i.ha^{-1} eliminated *C. amoena* from the communities, whereas the other three species were much less sensitive [9]. When used for pasture weed control, AP at a soil concentration of 0.2 μg.kg^{-1} exhibited crop injury ratings for bell pepper, eggplant, tomato, muskmelon and watermelon of 48, 67, 71, 3 and 3%, respectively, with fruit yield losses relative to the untreated control being 61, 64, 95, 8 and 14% [10]. As a systemic herbicide, AP is absorbed by the foliage and roots of actively growing plants and translocated to the meristematic (high-growth rate) areas of the plants, including the roots [11–13]. It interferes with plant growth metabolic pathways, affecting the growth process [14]. The hydrophobicity of the leaf surface affects the efficiency of the herbicide, which suggests its penetration through the cuticle, but with some plants, the effect of stomatal activity can also be included [15].

AP has a high potential to run off into surface water or leach into the soil profile and groundwater [16]. The half-life of AP in the environment was shown to be 6 to 500 days, varying according to the soil type, rainfall amount, and soil temperature [17,18]. The breakdown of AP by sunlight forms malonamic and oxamic acids and acid amines. In the environment, AP dissociates to its anionic form, which makes the compound highly soluble, non-volatile and exhibiting low adsorption to soils [19].

The toxic dose of AP in humans and animals is relatively high, and the herbicide is classified in the toxicity category IV [8]. AP has not been found to have carcinogenic or mutagenic effects, except for the in vitro chromosome aberration assay utilizing rat lymphocytes. The estimated environmental concentrations (EEC) for chronic exposure to surface and groundwater are 1.937 and 0.630 ppb, respectively. The values of dietary tolerances established for free and conjugated residues in crop and food commodities are as follows: wheat grain: 0.04 ppm, cattle fat and meat: 0.02 ppm and milk: 0.03 ppm [20]. The modern concept of micropollutants has broadened the perception of environmental pollution and its impact on human health. Micropollutants such as endocrine disrupters and pesticides are present in trace amounts in environmental matrices. Humans are exposed to micropollutants via food, water, air and daily consumer products [21,22]. Small amounts of AP persisting in soil can be regarded as micropollutants, and their long-term effects on human health are unknown.

Even though the EPA memorandum reviewing ecological risk assessment for AP recommends not to use a plant material treated with AP as mulch material in the following 18 months [8], such a recommendation has not been strictly observed in the Czech Republic due to pooling of straw and insufficient control. Our aim was to investigate the transfer of AP from straw mulch treated with AP-containing Mustang Forte herbicide into strawberry plants and fruits. The release of AP from the straw mulch caused by rain and irrigation water during a commercial, three-year production cycle at a strawberry farm was measured. As the separation of AP from complex organic matrices is difficult [19], our research also included the development and optimization of a highly sensitive method enabling us to detect AP in wheat straw and plant tissues. The concentrations of AP in the roots, leaves and fruits of strawberry plants representing different years of the three-year production cycle were measured and compared with EEC values established by the EPA for water and food commodities [20]. The study also measured the effect of AP-treated straw mulching on the flowering and fruiting of strawberry crops.

2. Materials and Methods

2.1. Strawberry Plant Growth Experiments

2.1.1. Plant Material

Two strawberry (*Fragaria* L.) varieties often used in Czech strawberry farms were used in the study, Karmen and Elsanta. They were provided by Farm Hanč (Vraňany, Czech Republic). The former is a medium–early variety whose fruits are large, kidney-shaped and dark red-colored. It was registered in the Czech Republic in 1971 [23]. The latter variety is of Dutch origin and was registered in the Czech Republic in 1994. It is an early variety with a long harvesting period (May–June) and large aromatic fruits that provide large yields [24].

2.1.2. Pot Experiments Investigating the Effect of Aminopyralid on Strawberry Flowering and Crop

Strawberry plants were grown in 5 L round pots in a special substrate used for strawberry production (AGRO CS a. s., Říkov, Czech Republic). The strawberry plants were put in the soil in the pots on 24 April 2022. Two weeks after planting, the straw mulch was added. The irrigation was 2 L of water per container 2–3 times weekly. The Karmen and Elsanta cultivars were used in the experiment. The following parameters were evaluated: total number of flowers per plant, total number of ripened fruits per plant and total mass of the harvested fruits per plant [25,26]. The assessment of the above parameters took place over the period of 16 May to 18 August 2023, two times a week.

Wheat straw used for mulching was treated with Mustang Forte herbicide (Corteva Agriscience Czech s.r.o., Prague, Czech Republic) at a regular dose of 1 L.ha^{-1} in the field. The non-treated straw was used as the control.

2.1.3. Analysis of Commercial-Farm, Strawberry Production Showing Translocation of Aminopyralid

The samples were collected at a commercial strawberry plantation (Farm Hanč, Vraňany, Czech Republic) to span a three-year production cycle. The sampled field represented an area of 450 m^2. Strawberry plants (Karmen cv.) were planted into sandy soil loam (pH 6.9) at different plantation plots in the spring of 2019, 2020 and 2021 and were growing in the presence of wheat straw mulch (10 cm layer). In 2020 and 2021, a fresh layer of wheat straw was put on the old, weathered mulch layer in the spring. At the time of strawberry harvest in June 2021, the samples of plant roots, leaves and fruits were collected together with the corresponding samples of the mulch layer at the three above plantation plots. Thus, the mulch samples represented one layer (2021), two layers (2020) and three layers (2019) of the straw mulch. Consequently, the plant part samples represented roots, leaves and fruits of three-, two- and one-year-old plants. However, all the fruits were from the 2021 crop. Average precipitation and temperature were recorded during the three-year experiment and are shown in Supplement File S1, Table S1. Three replicates were used throughout the experiments.

2.2. Determination of Aminopyralid

2.2.1. Sample Preparation

Extraction of AP from a straw material was optimized using acidified, non-buffered liquid/liquid extraction using a salt mixture of QuEChERS Bond-Elute (Agilent Technologies, Inc., Santa Clara, CA, USA).

Briefly, the aliquot of 1.0 g straw was wetted with an aliquot of 10 mL of Milli-Q water in the 50 mL centrifuge tube at +4 °C for 30 min. Then, 10 mL of acidified acetonitrile (1% formic acid *v*/*v*) and, subsequently, QuEChERS salt mixture (4 g $MgSO_4$ and 1 g NaCl, Bond Elute, Agilent) were added. After centrifugation (11,000 rpm, 4 °C for 10 min), an aliquot of 1 mL extract was evaporated under a stream of nitrogen to dryness at 40 °C. The dry sample was dissolved in 1 mL of acidified methanol (1% formic acid) and centrifuged (11,000 rpm, 4 °C for 5 min) before analysis. For straw samples, no further clean-up was applied.

Plant samples (roots, leaves and fruits) were extracted similarly to the straw samples, and further, an aliquot of 1 mL acetonitrile extract was cleaned up with 30 mg of graphitized carbon black (GCB) [27]. The cleaned extract was evaporated to dryness by nitrogen, dissolved in acidified methanol and centrifuged before LC/MS/MS application.

2.2.2. Analytical Conditions

The analyses were performed with AB Sciex LC QTRAP 6500+ MS/MS system with a TurboV source (Framingham, MA, USA) equipped with a Shimadzu Exion HPLC (Kyoto, Japan) using multiple reaction monitoring (MRM) and ESI+ ionization mode. Detailed MS settings are given in Supplement File S3 (Table S3). AP was separated at 30 °C on Ace Excel 2 Super C18 column (100 × 2.1 mm, 3 μm, 90 Å) with a gradient elution of mobile phases using Milli-Q water with 0.1% formic acid (A) and methanol (B). Gradient program started with a 50:50 (*v*:*v*) AB mixture for 0.5 min and increased linearly to 100% of B in 6 min; from 6 min to 6.5 min, gradient elution returned to initial conditions of 50:50, and the system was equilibrated with the initial conditions for 3.5 min. The flow rate was set at 300 $\mu L.min^{-1}$, and the injection volume was 2 µL. The method of AP extraction for straw samples was validated on the basis of SANTE/12682/2019 guidelines [28]. For validation details, see Supplement File S4.

2.2.3. Data Processing

The mass spectra data obtained from the analysis were processed with Analyst 1.7.1 (AB Sciex, Singapore). Comparison of mean values was subjected to one-way analysis of variance (ANOVA) and Tukey's post-hoc test at 5% level of probability ($p < 0.05$) using statistical software IBM SPSS 28.

2.2.4. Chemicals and Reagents

Analytical standards of AP (CAS No:150114-71-9), methanol and acetonitrile for extraction and chromatography elution were all obtained from Honeywell (Fluka, Germany).

3. Results

3.1. Assessment of Aminopyralid in Straw Mulch

AP concentration was determined in mulching straw samples with three different particle sizes applied within three years. The validation parameters of the used LC/MS/MS analytical method were set according to EURL [29]. Validation scores for the method are presented in Supplement File S2 (Table S2). The limit of quantification of the AP extraction method from straw matrices was determined as the lowest spiked concentration level of 5 $ng.g^{-1}$, which met the criteria of trueness (70–120%) and precision (<20%). The coefficient of determination (R^2) for the calibration curve was higher or equal to 0.9939. Simultaneous analysis with powdered straw samples from each sampling year was conducted with particle sizes of 0.5–1.5 mm to ensure that the extraction yield was not affected by the particle size (Table 1). The tested samples of straw treated with Mustang Forte herbicide were removed in individual years and showed different contents of AP. The results document large differences between the levels of AP in various straw materials, demonstrating heterogeneity resulting from the individual treatment events (Table 1).

Table 1. Content of aminopyralid in Mustang Forte-treated straw samples based on particle size.

Year/Straw Size (cm)		Aminopyralid ($ng.g^{-1}$)	Std. Deviation	RSD%
2019	0.5	6.7867 ^{a}	1.75	26%
	1.0	5.3222 ^{a}	0.75	14%
	1.5	5.2380 ^{a}	1.48	28%
2020	0.5	15.5070 ^{b}	0.91	6%
	1.0	16.2352 ^{b}	0.29	2%
	1.5	19.0908 ^{c}	0.52	3%
2021	0.5	31.1671 ^{d}	8.96	28%
	1.0	22.7486 ^{d}	4.58	20%
	1.5	22.3150 ^{d}	1.50	7%

$^{a, b, c, d}$ Superscripts are statistically significant differences according to Tukey's honest significant difference test.

3.2. Effect of Aminopyralid on Strawberry Flowering and Fruit Crop

The effect of Mustang Forte residues leached from wheat straw treated with the herbicide on the flowering and fruiting of strawberry plants using two strawberry cultivars, Elsanta and Karmen, was monitored (Figure 1). The experiments were conducted in pots under conditions simulating maximally those prevalent during the production cycle at a strawberry farm. The pot experiments enabled us to observe strawberry plants closely during both the flowering and fruit-formation periods. The statistically significant effects were the following: a decreased number of flowers, a decreased number of ripened fruits, and a decreased total weight of ripened fruits. The values in the diagrams are expressed per strawberry plant. The effect was cultivar-dependent, and the Karmen cv. was more sensitive than the Elsanta cv. (Figure 1).

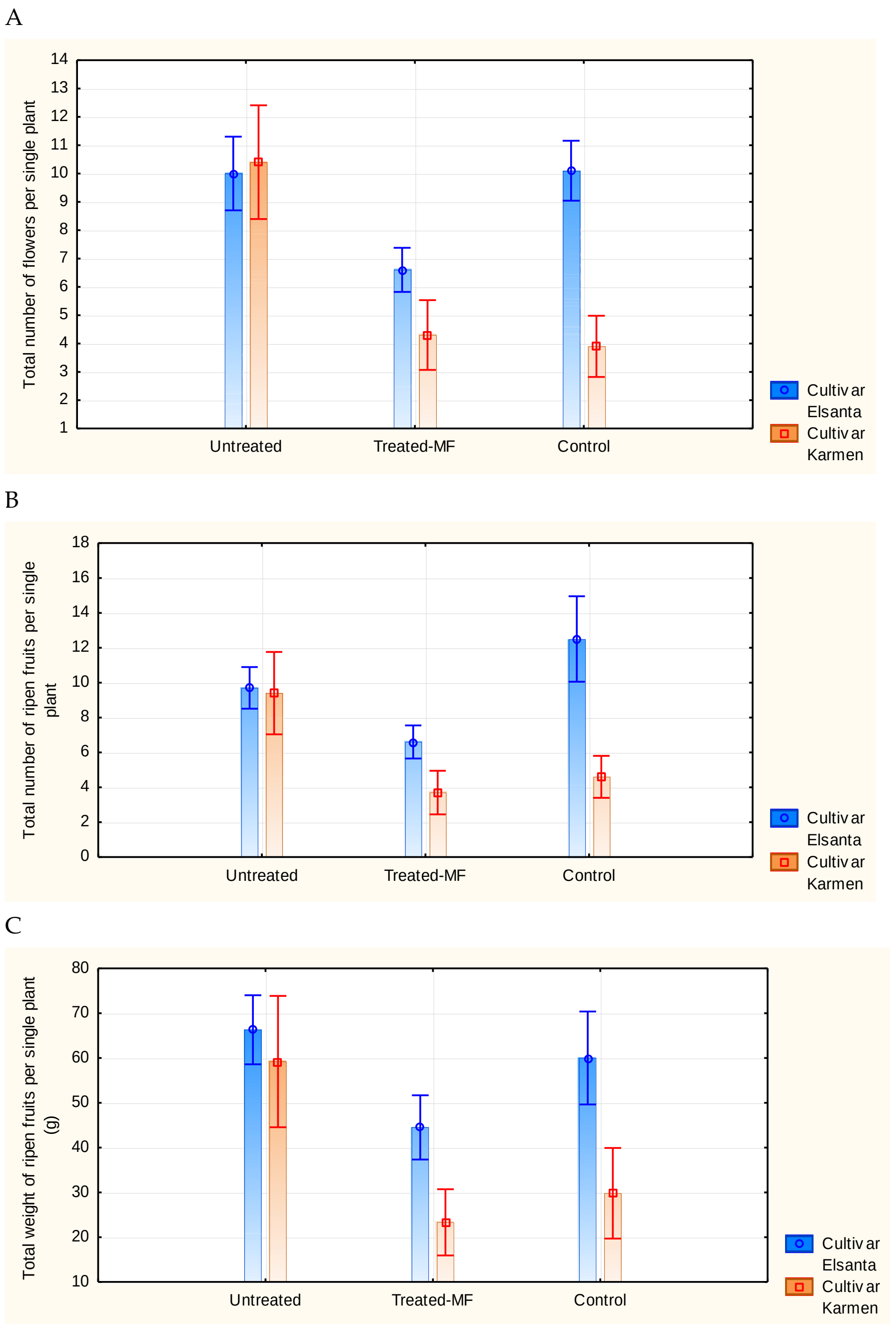

Figure 1. Effect of wheat straw treated with Mustang Forte on flowering and fruiting of strawberry plants expressed as total number of flowers per single plant (**A**), total number of ripened fruits per single plant (**B**) and total weight of ripened fruits per single plant (**C**). Mulching with straw treated with Mustang Forte, Treated MF; mulching with straw not treated with Mustang Forte, Untreated; no mulching used, Control. Strawberry cultivars Elsanta and Karmen were used.

The effect of mulching itself was also demonstrated by comparing the situations where strawberry plants were mulched with a non-treated straw and where no mulching was applied (Figure 1). In the Karmen cv., the mulching increased the number of flowers and fruits, including the total weight of fruits. In the Elsanta cv., the effects were not statistically significant (Figure 1).

3.3. Translocation of Aminopyralid from Straw Mulch to Strawberry Plant Tissues

The purpose was to measure how much AP was taken up by growing strawberry plants and how the herbicide was subsequently distributed in various parts of the plants including the fruits. We analyzed the content of AP in the straw that was used as the straw mulch during the three-year production cycle of strawberry fruits and tried to correlate the content of AP in the mulch layer with that in the strawberry plant tissues. A three-year production cycle represents a harvest of strawberries from the plantation once a year for three consecutive years. A new mulch layer was added to the plantation once a year, at the beginning of the growth period. The collected samples always represented the whole profile of the mulch layer surrounding the plants. As a result, the samples removed in 2021 from the three plantations established in the years 2019, 2020 and 2021 represented three, two and one mulch layer, respectively (Table 2).

Table 2. Aminopyralid content in mulch straw from plantations measured in 2019, 2020 and 2021.

Mulching	Aminopyralid (ng.g^{-1})	Std. Deviation	RSD%
Three layers (2019)	1.6150 ^{a}	0.26	16%
Two layers (2020)	19.3325 ^{b}	1.82	9%
One layer (2021)	9.0566 ^{c}	1.02	11%

$^{a, b, c}$ Superscripts are statistically significant differences according to Tukey's honest significant difference test.

There were significant differences in the content of AP in the mulch straw samples removed in the individual years of the production cycle. This can be explained by the different contents of AP in the batches of the straw harvested in the field that represented different herbicide treatment events and also by biotic and abiotic processes that occurred in the mulch during the time and could affect the release of AP from the straw matrix. The higher concentrations of AP in the mulch in 2021 and 2020 may reflect an increase caused by a loss of straw material mass due to decomposition similar to that in compost but probably less intense because of the absence of a thermic phase. When we measured the content of AP in wheat straw during the composting process (Supplement File S1, Figure S1), a three-fold rise in AP concentration was observed within the first three weeks, and then the value did not change much for the next twelve weeks. On the other hand, the low content of AP measured in the 2019 straw sample may indicate an increased release of AP in the third year attributable to both biotic and abiotic processes taking place in the mulch layer. The differences in the content of AP in the mulch did not, however, correlate with the content of AP in plant parts shown in Figure 2.

The absorption of AP from SM via soil to the roots and above-ground parts of strawberry plants was investigated (Figure 2). The concentrations detected in strawberry roots, leaves and fruits showed that the highest accumulation of AP was in fruits, where the values reached about 1.2–1.3 ng AP.g^{-1} dry mass. Those values were similar for the fruits harvested in all three years. The lowest concentrations were detected in roots, where they were sometimes below the detection limit (Figure 2). No large fluctuations in AP levels in plant parts were observed between the individual years in contrast to the AP concentrations present in SM (Figure 2, Table 2).The lower accumulation of AP in the roots, compared to the leaves and fruits, highlighted the roots' role as a pipeline conveying solutes to more metabolically active plant parts, whose tissues probably had more structures capable of binding AP than the root.

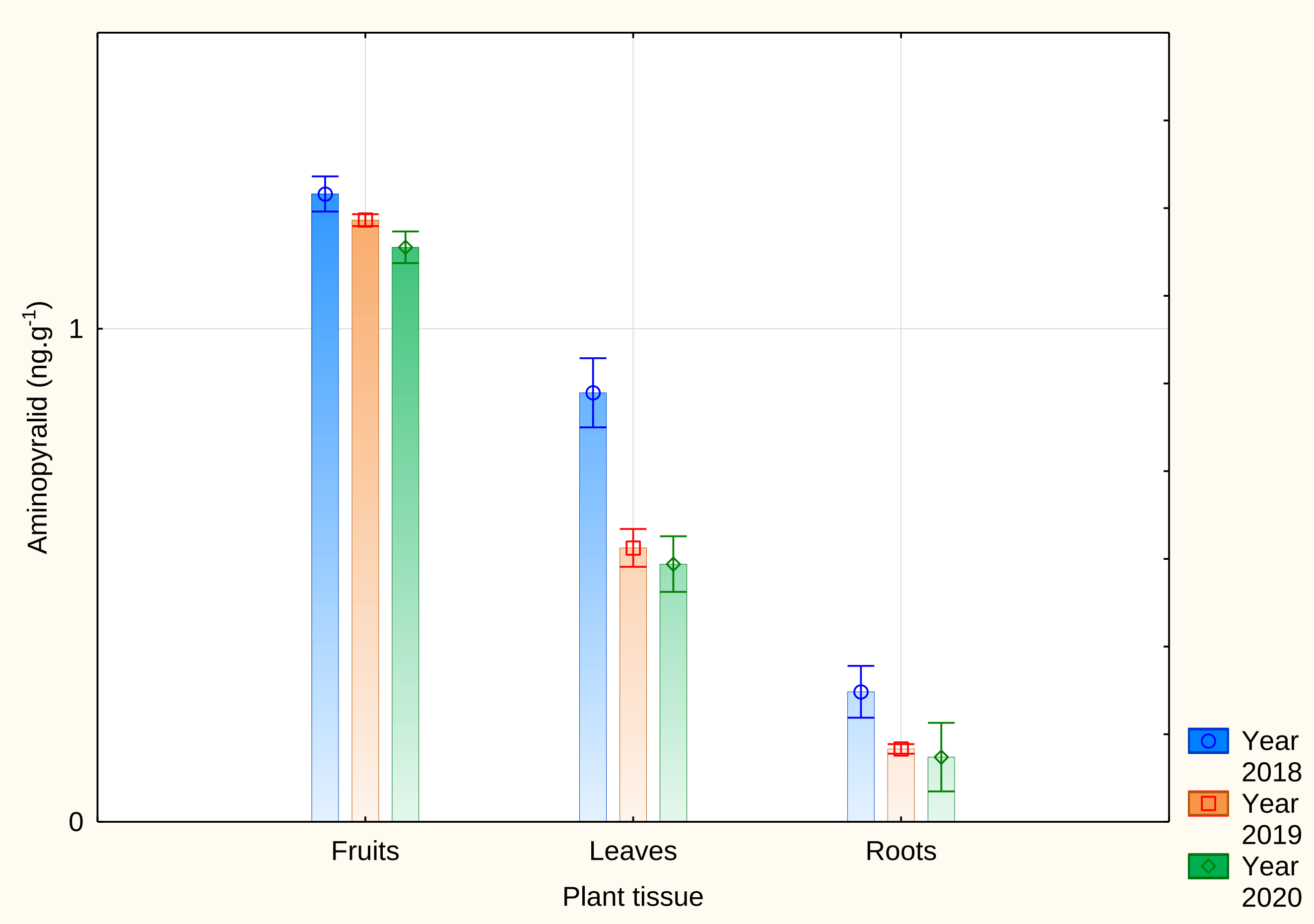

Figure 2. Aminopyralid content of strawberry plant (Karmen cv.) tissues expressed per g of dry biomass in a three-year production cycle.

4. Discussion

4.1. Development and Validation of the Extraction Method

An acidified extraction solvent was introduced by EURL [29], using 1% formic acid containing acetonitrile as the extraction solvent in acidic pesticide analysis. Although AP is an acidic herbicide derivative of picolinic acid, it is not included in strict acidic pesticides. As a result, the acidified extraction solvent helped to extract AP and maintain stability. The solvent exchange of the crude extract from acidified acetonitrile to more LC-MS/MS friendly 1% formic acid containing methanol improved the signal quality of AP as the clean-up step. To the contrary of several published methods [28,30], more than 2 μL of the injection volume significantly increased the background effect and masked the ions; therefore, the injection volume was set at 2 μL, of which the matrix effect was almost 80% that of solvent-based external calibration standards.

The studies of AP residues on different commodities provided lower LOQ values, particularly molecular imprinted particle cartridge-based methods: 0.77 μg.kg^{-1} and 0.41 μg.L^{-1} in milk samples [31]. According to SANTE/12682/2019 guidelines [28], the LOQ is 10 μg.L^{-1}. However, the most similar research study on plant-based food commodities provided a comparable LOQ of 50 μg.kg^{-1}, which is 10 orders of magnitude higher [26].

SANTE/12682/2019 guidelines recommend milling the samples, preferably to obtain particles smaller than 1 mm that are used for extraction [28,32]. Our results suggest that the size of straw particles was not statistically significant with respect to the amount of AP extracted from the straw treated with Mustang Forte (Table 1).

4.2. Effect of AP on Flowering and Fruit Crop

AP has been widely used to suppress broadleaf weeds in the production of cereals and in pastures and grasslands [11,12], but its negative effects on various crops have also been documented. At a concentration of 0.2 μg.kg^{-1} soil, AP caused significant crop injury and fruit yield losses in pepper, tomato and eggplant production [10]. Potato tuber production was reduced at rates of 15 g AP.ha^{-1} [33]. Soukupová and Koudela [34] described a 94% injury to tomato plants at a dose of 15 g AP.ha^{-1}, but the plant height was already negatively affected at a concentration of 3 g AP.ha^{-1} and deformation of germinating seeds was observed at 0.6 g AP.ha^{-1}. Mustang Forte is a mixture of compounds containing 180 g 2,4-D, 10 g aminopyralid and 5 g florasulam per liter. According to the estimated half-lives for the three herbicide compounds, AP is the most persistent of them [17,18,35,36]. Therefore, we attribute the effect mainly to AP, whose major translocation from the herbicide-treated straw via the soil matrix to the strawberry plants was documented. In our experiments, we determined the amount of AP in the mulch straw that was subsequently leached by rain and irrigation water into the soil and further accumulated in strawberry plant tissues. The effects of AP observed included a decrease in the number of flowers, ripened fruits and the total weight of the strawberry crop (Figure 2). The effect was cultivar-dependent; the Karmen cv. was more sensitive than the Elsanta cv. (Figure 2). The reason for the higher sensitivity of Karmen cv. is not known.

4.3. Translocation of Aminopyralid from Straw Mulch to Strawberry Plants and Fruits

When the straw of wheat treated with AP in the field is used for mulching, a significant extraction of organics from the straw material, including the herbicide, takes place due to water leaching, and the compounds reach the soil and water [6,7]. Here, wheat straw containing AP due to the application of a Mustang Forte herbicide mixture was used for mulching strawberry plantations. Our results documented the effective leaching of AP from the straw mulch and its translocation into strawberry plants, including the fruits (Table 2, Figure 2). Little is known about the absorption and translocation of AP in plants [8], and, to our knowledge, no information exists on the translocation of AP in strawberry plants. Clopyralid was found to be more readily absorbed and had a greater association with both roots and shoots in Canada Thistle (*Cirsium arvense*) than AP. This difference in translocation was not explained, and the authors pointed out that the values of log Kow and pKa were very similar for both compounds [11].

The detected contents of AP in strawberry fruits were close to the values of the EEC for chronic exposure to groundwater and surface water established by the EPA, of 0.630 and 1.937 ppb, respectively. At the same time, they were one order of magnitude lower than the dietary tolerances given by the EPA for wheat grain and cattle milk and meat [20]. Consequently, the imminent health risks for the consumption of strawberries resulting from the mulching of strawberry plantations with straw treated with AP seem to be low. However, auxin and auxin-like compounds such as 2,4-D were found to affect the transcription process, inducing strong G1 arrest in cancer cells and regulating tumor morphology in tobacco plants [37,38]. Recent research revealed that long-term exposition to low concentrations of problematic, endocrine-disrupting micropollutants, such as various pharmaceuticals, antibiotic residues and pesticides, can pose a serious and unpredictable health risk for humans when the compounds are introduced into the soil or groundwater, e.g., [39–42].

5. Conclusions

AP has been widely used to control broadleaf weeds and woody plants. Due to its persistence and mobility in the environment, it contaminates soil, water and agricultural waste materials such as straw, manure and compost. After harvest, AP-treated and untreated wheat straw is often pooled, stored and, subsequently, used for mulching. As a result, AP can reach the strawberry production cycle. Monitoring of the translocation of AP from herbicide-treated straw to strawberry plant tissues and fruits documented the accumulation of AP in strawberry fruits at levels that were about 20 times lower than the dietary tolerances given by the EPA for common food commodities. The amounts of AP in strawberry fruits were similar during the whole three-year production cycle, irrespective of the various concentrations of AP in the mulch straw. The use of AP-contaminated straw for mulching significantly decreased the crop yield. The results quantify the effects of using straw treated with auxin and auxin-like herbicides for mulching on strawberry crops and the contamination of fruits with AP. Because of the wide use of auxin and auxin-like herbicides in agriculture, further research on the persisting concentrations of those herbicides and their residues in soil and on their long-term effects on the human organism is needed. Precautions against using herbicide-contaminated straw for mulching should be taken in horticulture to eliminate herbicides from the production cycle.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9111192/s1. Supplement File S1: Table S1: Temperature and precipitation characteristics of strawberry plantation (Farm Hanč, Vraňany, Czech Republic); Figure S1. Time course of changes in the content of aminopyralid in Mustang Forte-treated wheat straw during composting process expressed per dry biomass; Supplement File S2: Table S2. Validation results of straw samples (n = 3); Supplement File S3: Parameters of MS analysis of aminopyralid; Supplement File S4: Analytical method validation.

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Data Availability Statement: The data presented in this study are available in this article and its Supplementary Materials here.

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