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## Dynamics of herbicides degradation in carrot (*Daucus carota* L.) roots and leaves

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**Abstract:** This research had two main aims. First, to analyse the degradation dynamics of herbicides commonly used in carrot (aclonifen, clomazone, flufenacet, linuron, metribuzin, pendimethalin, S-metolachlor). Second, to compare the amount of herbicide residues with the maximum residue level and with requirements of non-residual production. The field experiments were conducted in 2012–2016. All tested herbicides resulted in relatively low concentrations of residues in carrot roots (up to 10 µg/kg) when the recommended withdrawal period was followed between application and harvest. The concentration of S-metolachlor in carrot roots exceeded the maximal residual limit (MRL) if the application was carried out four days before harvest. The measured values of other tested herbicide residues in carrot roots did not exceed the MRL in any of the tested samples. Pre-emergent use of clomazone, linuron and flufenacet could be recommended for non-residue carrot production. Post-emergent use of metribuzin can be used for non-residue carrot production if the interval between application and harvest is at least 80 days. Concentrations of herbicide residues in carrot leaves were many times higher than in roots. All tested herbicides can be applied for safe carrot production if applicators adhere to the requirements for use.

**Keywords:** vegetable; pesticide; contamination; non-residue production; weed control

Carrot (*Daucus carota* L.) is one of the most widely grown and important vegetables (Welbaum 2015), and carrot production involves farmers of many socioeconomic levels (Araújo et al. 2016). The total world production of carrots was 43 million tons. The total world production area of carrots is roughly about 1.47 million ha, and the average yield 37 t/ha (FAOSTAT 2017). The competitive ability of carrots is low because the emergence and early growth are relatively slow. Without sufficient control, weeds may cause a yield loss up to 94% (Coelho et al. 2009).

Several herbicide options have been registered for weed control in carrots. Pre-emergent (PRE) herbicides for use in carrots include pendimethalin, aclonifen, clomazone, prometryn, trifluralin, flufenacet, metribuzin, and dimethenamid (Malidža et al. 1997, Ogbuchiekwe et al. 2004). Similar soil active

herbicides can be used post-emergence (POST): linuron, metribuzin, prometryn, flufenacet, pethoxamid, S-metolachlor, flumioxazin, oxyfluorfen, metoxuron, chlorpropham, ioxynil and others (Malidža et al. 1997, Ogbuchiekwe et al. 2004, Kavaliauskaite et al. 2009, Robinson et al. 2012). In the European Union, however, many of these herbicides have recently been restricted, and others are likely to follow in the near future. As a consequence, fewer herbicides will be available for weed control and most of them at a lower application rate.

In a global market, safety standards of edible produce are a worldwide concern; however, the responsibility often falls on the growers to balance agronomic practices with market standards. The concentration of pesticide residues in vegetable below the maximal residual limit (MRL) rarely represent a toxicological

risk (Winter 1992). Unfortunately, the growers might not know if the herbicides registered for the crop will result in excessive residues for selling their produce.

Because of the specific behaviour of different herbicides in plants and soil, it can be assumed that amounts of their residues in harvested carrots are different. Therefore, the first objective of this study was to compare the degradation dynamics of residues of most often used herbicides in carrots to MRL of tested herbicide. The second objective of this study was to develop recommendations for herbicide weed control for low-residual and non-residual carrot production. Low-residual production is the agricultural production, in which the crop protection is carried out so that residues of used pesticides in harvested products are below the limit for a predetermined action threshold, for example, 25% MRL or 50% MRL (Kocourek et al. 2017, Horská et al. 2020). More restrictive about the amount of residue is non-residue production, where residues of used pesticides in products are below the limit of 10 µg/kg. This limit is currently used worldwide for products intended as infant food and is strictly monitored by Commission Directive 2006/141/EC. To our knowledge, there is a lack of studies regarding herbicide degradation in plant products, and this is probably the first study on herbicide degradation in carrots.

## MATERIAL AND METHODS

Small plot field trials were carried out in carrot (cv. Grivola) in Demonstrational and Experimental field of Czech University of Life Sciences Prague in the Czech Republic (286 m a.s.l., 50°7'N, 14°22'E) in 2012–2016. The soil was classified as Haplic Chernozem with a clay content of 19%, sand content of 25%, silt content of 56%. These contents are specific for silt loam soil. Sorption capacity was

212 mmol<sub>+</sub>/kg, and soil pH<sub>KCl</sub> was 7.5. The region has a temperate climate with an annual long-term air temperature of 9.2 °C and total annual long-term precipitation of 510 mm. The potato was the previous crop in all experimental years. None of the tested herbicides were used in the previous crop.

All plots were arranged in randomised blocks of 13.5 m<sup>2</sup>. Double (15 cm) row spacing was 75 cm, and in-row plant spacing was approximately 4 cm. The carrot was sown on 4, 29, 7, 21, and 28 April 2012, 2013, 2014, 2015 and 2016, respectively. Common agriculture practice by the European and Mediterranean Plant Protection Organisation was used according to carrot requirements. Tested herbicides (Table 1) were applied by Schachtner non-residue sprayer at a spray volume of 300 L/ha and pressure of 0.2 MPa. The highest registered rates of all tested herbicides were used. Herbicides were applied PRE (second day after sowing) and POST (Table 2).

The samples of carrots were collected continuously during the growing season from the central part of each plot. The first sampling was carried out when the diameter of top root parts achieved 10 mm on average. There was a two-week interval between first and second sampling and between second and third sampling. A minimum of four carrots was collected from one plot at each sampling term. Roots of carrot were collected at each sampling time. In 2014–2016, carrot leaves were also collected in plots after POST herbicide application. The samples were stored at –20 °C until the extraction procedure.

All harvested carrot samples were tested in the fully certified laboratory of the Department of Food Analysis and Nutrition at the University of Chemistry and Technology Prague using the LC-MS/MS method accredited according to the EN ISO/IEC 17025 standard. The analytical method used in this study

Table 1. Description of tested herbicides

Trade name	Active ingredient (a.i.)	Formulation	Content of a.i. (g/L)	Application rate (g/ha a.i.)	Manufactured
Bandur	aclonifen	SC	600	1 800	Bayer CropScience
Stomp 400 SC	pendimethalin	SC	400	1 200	BASF
Afalon 45 SC	linuron	SC	450	500	ADAMA
Sencor Liquid	metribuzin	SC	600	300	Bayer CropScience
Cadou 500 SC	flufenacet	SC	500	150	Bayer CropScience
Dual Gold 960 EC	S-metolachlor	EC	960	1 152	Syngenta
Command 36 SC	clomazone	SC	360	72	FMC corporation

SC – soluble concentrate; EC – emulsifiable concentrate

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Table 2. Terms of post-emergence application in experimental years

Herbicide	Days after sowing				
	2012	2013	2014	2015	2016
Aclonifen	68	44	65	57 and 70	61
Pendimethalin	–	–	–	–	49
Linuron	68 and 84	44 and 69	65 and 77	48 and 70	35 and 61
Metribuzin	68, 76 and 96	44, 56 and 77	65, 70 and 91	48 and 70	35 and 61
Flufenacet	68	44	65	–	49
S-metolachlor	84	69	77	70	35 and 61

is based on EN standards. The determination of pesticide residues was done by the QuEChERS method that has been readily accepted by pesticide residue analysts. Pesticides were extracted from a portion of a homogenised sample (10 g) by acetonitrile. After separation of aqueous and acetonitrile layers (induced by addition of anhydrous  $MgSO_4$  and NaCl salts), an aliquot of the upper organic layer was transferred into a vial for LC-MS/MS. A Ultra-High Performance Liquid Chromatography system, coupled to a triple quadrupole mass spectrometer, with electrospray ionisation in positive ion mode (ESI+) was used for the final identification and content of herbicides residues. The generated data were processed using MassLynx software, version 4.1 (Waters Corporation, Milford, USA). The method used for residues analysis was fully validated in line with the requirements stated in the European Commission's guidance document SANTE/12682/2019. The limit of quantification was 2  $\mu g/kg$  for all analysed herbicides. The measured values were compared with the MRL established by Regulation (EC) No. 58/2019 and with requirements of non-residual production. The limit for non-residue production is 10  $\mu g/kg$  of herbicide in harvested carrots regardless of the active ingredient.

The obtained data were processed in R project, version 3.6.1 (R Core Team, 2019) and subjected to

the comparison analysis (*t*-test) to reflect the differences in experimental years. Non-linear models of degradation of individual herbicides in carrot were calculated using the exponential decay formula in drc package using the following equation:

$$y = a \times (\exp(-x/b))$$

where: *y* – amount of active ingredient ( $\mu g/kg$ ); *x* – number of days after herbicide application; parameter *b* > 0 – determines the steepness of decay.

The goodness of fit was assessed by *F*-test. All tests were performed on the significance level of 0.05. Parameters of models and the analytical results are shown in Table 3.

## RESULTS AND DISCUSSION

In literature, the availability of information about the herbicide residues in the plant product and herbicide uptake by carrot is scarce; therefore, this work can be considered as an innovative approach.

In this study, many active ingredients were tested. The different properties of each herbicide affect the uptake, translocation and soil persistence and will, in turn, have an impact on the herbicide residue found in the plant product. The herbicide residue levels in carrots from individual years were similar, and data combined across years

Table 3. Parameters of exponential decay models

Active ingredient	<i>a</i>	CI (95%)	<i>b</i>	CI (95%)	<i>F</i> -test	<i>P</i> -value
Aclonifen	60.52	45.9–75.1	31.14	22.6–39.6	0.98	0.541
Pendimethalin	165.73	121.4–210.0	22.37	16.8–27.9	0.89	0.646
Linuron	76.53	48.5–104.6	9.09	5.6–12.5	1.86	0.290
Metribuzin	21.40	16.8–27.1	9.28	6.1–12.4	0.78	0.147
Flufenacet	129.63	83.2–156.1	13.82	8.2–19.4	0.59	0.789
S-metolachlor	122.11	82.3–161.9	15.66	10.08–21.24	1.59	0.397

*a* – parameter attained at *x* = 0; *b* – parameter determining the steepness of the decay; CI – confidence interval; *P*-value = 0.05

revealed no statistical differences ( $P > 0.05$ ). Therefore, it was possible to merge data from all experimental years and calculate exponential curves.

Aclonifen belongs to the diphenyl-ether chemical family, which inhibits protoporphyrinogen oxidase; however, this particular chemical is unique. Recently, it was identified aconifen actually targets solanesyl diphosphate synthase (Kahlau et al. 2020). Residues of aconifen were detected in most of the samples treated by this herbicide. The most contaminated

sample was collected four days after POST application (shortest tested interval between application of aconifen and carrot harvest), where  $55 \mu\text{g}/\text{kg}$  of aconifen was detected in roots (69% MRL). Residues of aconifen ranged from 0 to  $30 \mu\text{g}/\text{kg}$  when aconifen was applied at least 40 days before harvest. A lower amount of residue (up to  $26 \mu\text{g}/\text{kg}$ ) was found after the PRE application; however, in 2014 and 2015, no residue of aconifen was detected. This can be explained by the fact that the target for aconifen is

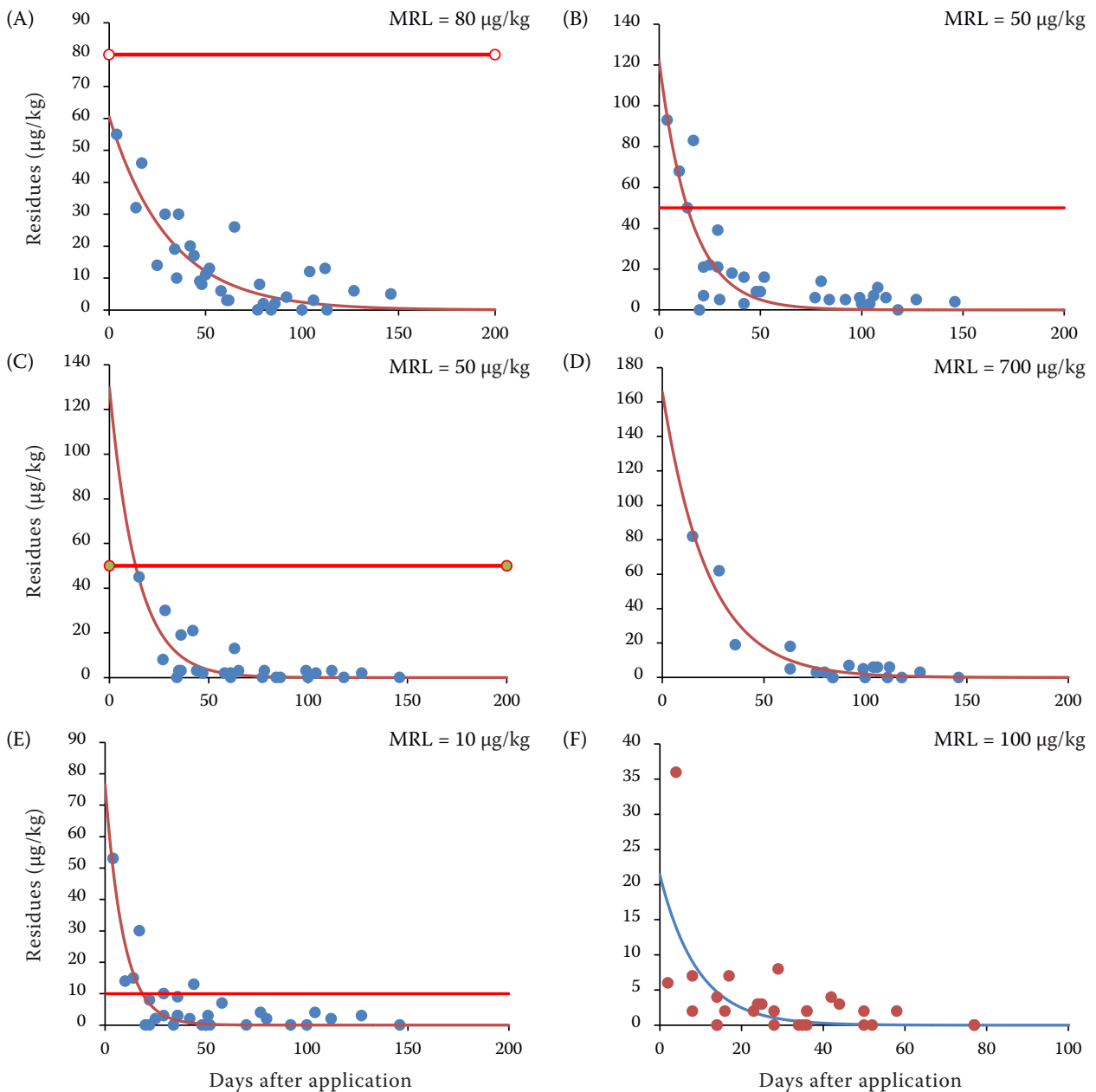


Figure 1. The course of degradation of (A) aconifen; (B) S-metolachlor; (C) flufenacet; (D) pendimethalin; (E) linuron and (F) metribuzin in carrot roots at time scale (data from 2012–2016). The red horizontal line shows maximal residual limit (MRL)

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clearly limited to the plant's upper parts (Kilinc et al. 2011). The dynamic of degradation of aclonifen in carrot roots is shown in Figure 1A, where the horizontal line shows MRL for aclonifen in carrot (80 µg/kg).

Residues of S-metolachlor were detected very often. In Pakistan, where S-metolachlor is a very common herbicide to control weeds in vegetable crops, detected residues were between 45–75 µg/kg (Amjad et al. 2013). A higher level of S-metolachlor residues can be caused by multiple applications at high rates in intervals of 2–3 days (Baig et al. 2009). If the S-metolachlor has been used POST, residues often exceeded the value of MRL (50 µg/kg). In 2012 and 2014, the S-metolachlor residues in carrot roots were relatively low (0–11 µg/kg). The most contaminated sample was collected four days after post-emergent application (shortest tested interval between S-metolachlor application and carrot harvest), where the detected amount of S-metolachlor was 93 µg/kg (186% MRL). The dynamic of degradation of S-metolachlor in carrot roots is shown in Figure 1B, where the horizontal line shows the MRL value. Much higher values of S-metolachlor residues were found in leaves. This active ingredient can be absorbed into the plant through the roots and shoots, but shoot tissues are generally more absorptive and the site of herbicidal activity (Wehtje et al. 1988).

Only very low flufenacet residues (0–3 µg/kg) were detected in carrot roots harvested from plots treated PRE by this herbicide. This is in accordance with the previous findings, which confirmed that primary uptake of flufenacet occurs both *via* the roots and emerging shoots of treated plants (Grichar et al. 2003). If flufenacet had been used POST, residues ranged from 0 to 45 µg/kg. No flufenacet residue was detected in 2014. The dynamic of degradation of flufenacet in carrot roots is shown in Figure 1C, where the horizontal line shows MRL for flufenacet in carrot (50 µg/kg). Similar results appeared when the flufenacet was applied in wheat, soybean, potato, and tomato (Imai et al. 2019).

Pendimethalin residue was found in all carrot root samples, which were harvested from plots treated PRE by pendimethalin (from 63 to 146 days after application) and ranged between 0 and 7 µg/kg (1% MRL). No residue of pendimethalin was recorded in 2014. A higher amount of residue (18–82 µg/kg) occurred after the POST application (harvest 15–63 days after application). However, detected values showed a large variation. Therefore, residues of pendimethalin less than 10 µg/kg cannot be guaranteed in specific soil and weather conditions. In different studies, pendimethalin and its metabolites were not detected

in any of the tested carrot samples (Engebretson et al. 2001, Singh et al. 2010). The dynamic of degradation of pendimethalin in carrot roots is shown in Figure 1D. The residues of pendimethalin were detected in leaves after PRE and POST application, as well. This is not completely in compliance with previous conclusions, which claimed that pendimethalin is absorbed by the roots and leaves but not translocated in the plant (Appleby and Valverde 1988, Gilliam et al. 1993).

Linuron was completely restricted in European Union in 2019. Linuron residue was found in all carrot root samples from PRE treatment (from 80 to 146 days after application) and ranged between 0 and 4 µg/kg (2% MRL). The residue of linuron in carrot roots fast decreased below MRL. The amount of linuron residue did not exceed 15 µg/kg after POST application in 2012–2015; however, in 2016, residues linuron ranged between 0 and 53 µg/kg (harvest was carried out 4–52 days after application). Khan et al. (1976) did not detect linuron residue at the final harvest time. In 2012, however, the small amount of residue (3 µg/kg) was detected even 127 days after the application. This could be due to the slow degradation of linuron in the soil during dry conditions. This agrees with the research of Fryer and Kirkland (1970), who detected linuron residues at levels less than 5 µg/kg in carrots sampled 15 weeks after the application of herbicide, and this residue amount was also affected by weather conditions. On the contrary, Løkke (1974) did not find any interaction between the amount of linuron residues (including metabolite products) in carrots and the interval between the herbicide application and carrot harvest (57–117 days). In our study, the dynamic of degradation of linuron in carrot roots is shown in Figure 1E, where the horizontal line shows MRL for linuron in carrot (10 µg/kg). Linuron residues were found in leaves in 2015 and 2016. This is expected because this active ingredient moves apoplastically with the transpiration stream after uptake by roots (Ducruet 1991).

Metribuzin was degraded under the value for non-residue production already two days after POST application in 2013. Metribuzin showed similar results with rapid degradation in sugarcane (Selim and Naquin 2011). Slower degradation of metribuzin was recorded in 2016, where 34 µg/kg of metribuzin was detected in carrot roots four days after POST application, which is still under the value of MRL (100 µg/kg). The residue of active ingredient metribuzin was not found in carrot roots harvested 70 days after POST application. The dynamics of degradation

Table 4. The concentration of herbicides ( $\mu\text{g}/\text{kg}$ ) in roots and leaves a month after application (mean  $\pm$  standard deviation)

Herbicide	2014		2015		2016	
	root	leave	root	leave	root	leave
Aclonifen	20 $\pm$ 11	217 $\pm$ 49	0 $\pm$ 0	87 $\pm$ 25	26 $\pm$ 12	194 $\pm$ 32
Pendimethalin	0 $\pm$ 0	9 $\pm$ 5	2 $\pm$ 2	62 $\pm$ 21	5 $\pm$ 3	18 $\pm$ 9
Linuron	0 $\pm$ 0	28 $\pm$ 14	3 $\pm$ 3	44 $\pm$ 26	3 $\pm$ 3	25 $\pm$ 11
Metribuzin	0 $\pm$ 0	34 $\pm$ 18	0 $\pm$ 0	61 $\pm$ 30	4 $\pm$ 2	54 $\pm$ 18
Flufenacet	0 $\pm$ 0	52 $\pm$ 20	2 $\pm$ 2	68 $\pm$ 24	3 $\pm$ 2	60 $\pm$ 26
S-metolachlor	0 $\pm$ 0	152 $\pm$ 47	5 $\pm$ 4	10 $\pm$ 5	16 $\pm$ 7	38 $\pm$ 15

of metribuzin in carrot roots are shown in Figure 1F. Low levels of herbicide residue ( $3 \mu\text{g}/\text{kg}$ ) were found in carrots up to 58 days after application. Therefore, it is necessary to respect the withdrawal period (60 days), which is, of course, important for all pesticides. A low decrease of metribuzin residues also reported by Stoleru et al. (2015), who detected low amounts of residue ( $2 \mu\text{g}/\text{kg}$ ) in vegetable samples at the harvest time. Moreover, the persistence of metribuzin in the soil could be greater than 60 days (Saritha et al. 2017).

Clomazone was applied only PRE because its metabolic selectivity to carrot is relatively low and cannot be used POST (Rigoli et al. 2008). The residue of clomazone was detected only in one sample ( $2 \mu\text{g}/\text{kg}$ ) of carrot root in 2012. In other experimental years, no clomazone residue was recorded in any tested sample of carrot. Low application rate ( $8 \text{ g}/\text{ha}$ ) and fast degradation of clomazone in the soil is probably the main reason for low concentrations found in carrot root samples in our study. Similar results were found in soybean, where the residue of clomazone at harvest time was always below the limits of quantification (Hu et al. 2011, Nalini et al. 2015). Residues of the clomazone were below the maximum residue levels in *Brassica napus* L. after 22 days after the herbicide application (Szpyrka et al. 2020).

Herbicide residue levels in carrot leaves were significantly higher compared to herbicide residues in roots (Table 4). This agrees with the research of Yajima et al. (2017), who founded the highest pesticide residue amounts in leaves. However, we did not detect any residue of clomazone in carrot leaves (only PRE application). Considering that carrot leaves are not usually eaten, this finding should not be concerning, and MRLs for carrots are only established for the roots.

The fate of the herbicide in the environment is affected by many factors; among them, temperature and moisture are most important. Differences in herbicide residue content in leaves in 2015 compared to 2014

and 2016 can be attributed to the low precipitation (sum April to August  $185 \text{ mm}$  in 2015,  $268 \text{ mm}$  and  $312 \text{ mm}$  in 2014 and 2016, respectively) and higher temperatures in this period. Clomazone half-life in the soil shortened significantly when the average temperature was higher and stable (Szpyrka et al. 2020). Periods of drought and low temperatures slow down the decomposition of herbicides in the soil and affect their uptake by plants (Grygiel et al. 2012).

In conclusion, the measured values of herbicide residues in carrot treated by aclonifen, pendimethalin, metribuzin, flufenacet and clomazone did not exceed the MRL in any tested sample. All tested herbicides can be used for low-residual carrot production if growers adhere to the recommended withdrawal period interval between application and harvest.

PRE application of clomazone, linuron and flufenacet can be used for non-residue carrot production. POST application of metribuzin can be used for non-residue carrot production if the interval between application and harvest is at least 80 days.

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