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Selectivity and efficacy of herbicides dimethachlor and pethoxamid in rocket crop

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Abstract: Field experiments were conducted to evaluate the efficacy, selectivity and health harmlessness of four application rates of two pre-emergent herbicides (pethoxamid and dimethachlor) in the rocket [*Eruca vesicaria* (L.) Cavanilles)]. Pethoxamid was found to be less efficient on the total weed density (efficacy 86.0–93.3%) in comparison with the effect of dimethachlor (94.9–95.8%). Dimethachlor expressed an excellent efficacy on *Echinochloa crus-galli* (L.) P. Beauvois, *Portulaca oleracea* Linnaeus, *Amaranthus retroflexus* Linnaeus, *Lamium purpureum* Linnaeus, and *Veronica persica* Poiret from the lowest tested application rate (800 g/ha). Pethoxamid showed an excellent efficacy on *E. crus-galli, Lamium purpureum*, *Lamium amplexicaule* Linnaeus, *V. persica*, and *P. oleracea.* In higher application rates, pethoxamid controlled *Chenopodium polyspermum* Linnaeus and *Chenopodium album* Linnaeus. In contrast to mostly negative effects of dimethachlor, pethoxamid showed either no effects or positive ones on the rocket yield. Residues of both herbicides in the harvested product were always below a 'default limit', which is the baseline maximum residue level for food. The selectivity of pethoxamid at an application rate of 960 g/ha was good, herbicide residues in the rocket were not detected and the yield of the rocket increased.

Keywords: *Eruca vesicaria* (L.) Cav.; maximum residue levels (MRLs); phytotoxicity; weed control

Rocket salad, arugula, or roquette [*Eruca vesicaria* (L.) Cavanilles)], belonging to the Brassica family (Brassicaceae), is one of the oldest crops traditionally grown in Southern Europe and Western Asia. Rocket is traditional in Mediterranean cuisine as a leafy green, but in recent decades it has been becoming more popular in Central Europe and in the United States. In the kitchen, the rocket is primarily used fresh in salads, pasta dishes, or cooked and eaten like spinach. Rocket is an annual, cool-season crop that flowers under long days and high temperatures. It is quick growing, being especially productive in spring, early summer, and autumn (Padulosi 1995; Doležalová et al. 2013).

Weeds are a major threat to most crops. In addition to the competition effect, weeds present in the field at harvest time can contaminate the rocket, especially when it is harvested mechanically (Holmstrom 2008). Any admixture in the harvested product may thus reduce product quality. Concerning the biological control of weeds, there are no effective methods available for the rocket crop. Hand weeding and mechanical cultivation are common practices but time-consuming and costly. To select an appropriate herbicide, it is necessary to know which weed species occur in the field. With respect to ensuring both effectiveness on the weeds and selectivity to crop, pre-emergent herbicides are more promising,

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because they control germinating weeds. Bensulide is the pre-emergent herbicide most commonly used in a rocket in Arizona and New Jersey (USA); it is effective against grass weeds and also controls some small-seeded dicot weeds. Sethoxydim and other ACCase inhibitors are post-emergent herbicides that control only grasses (Dimson 2001). Another option is to use non-selective herbicides with active substances such as carfentrazone-ethyl, paraquat dichloride, pelargonic acid, pyraflufen-ethyl, and glyphosate to control weeds in the field before the rocket sowing (Dimson 2001; Holmstrom 2008).

The problematics of the chemical control of weeds in the rocket crop have not been studied yet in the temperate climatic conditions of Central Europe. Since there is no pesticide registered for the rocket in the Czech Republic (Anonymous 2014), two herbicides which are registered in the Czech Republic for the related species (i.e. rapeseed and various cruciferous vegetables), were considered to test their efficacy, selectivity, and health harmlessness in the rocket. Both selected herbicides belong to chloroacetamides, which are categorized into the group 15 according to Herbicide Resistance Action Committee (HRAC) classification (HRAC 2010). They are absorbed by new shoots of seedlings and roots and a mode of their action is through the inhibition of lipid synthesis and cell division (VLCFAs inhibition), which inhibits weed germination (Böger et al. 2000; HRAC 2010; Yang et al. 2010; PPDB 2020). The effect of pethoxamid is systemic (PPDB 2020). Pethoxamid can be safely applied pre- and early postemergently in a wide range of arable and horticultural crops and controls a broad spectrum of grass and broad-leaved weeds effectively (Hunt et al. 2015; PPDB 2020). Dimethachlor is used pre-emergently to control most annual grasses and broad-leaved weeds (PPDB 2020).

Despite the benefits of pesticides for crop yields and their relevance for the economy, intensive and widespread pesticide use raises serious environmental and health concerns (Damalas & Eleftherohorinos 2011; Nicolopoulou-Stamati et al. 2016). Therefore, for each newly registered application of a pesticide for protection of a particular crop, it is necessary to be very thorough in setting an application rate that can be recommended to users as harmless to health, especially in leafy vegetables which have a short growing season. In order to limit the risks of presence of pesticide residues in foodstuffs, the maximum residue levels (MRLs) in or on food and feed of plant and animal origin were defined for selected pesticides throughout the European Union by the Regulation (EC) No. 396/2005. According to this regulation, MRLs are the highest levels of residues expected to be in the food when a pesticide is used according to the authorized agricultural practices. The regulation also established a general limit that applies in cases when no specific MRL has been defined for a particular crop (a 'default limit' of 0.01 mg/kg) as it is in the case of the rocket.

The present study aims (*i*) to evaluate the efficacy of four application rates of herbicides dimethachlor and pethoxamid, (*ii*) to compare the impact (i.e. the possible toxicity and influence on plant vitality and yield) of these herbicides on the rocket, and (*iii*) to evaluate the presence/absence of herbicide residues in the harvested product.

MATERIAL AND METHODS

Plant material and herbicide selection. The seed of rocket salad originating from the seed company Semo Ltd. (Smržice, Czech Republic) was used in the experiments. On the basis of a preliminary experiment in 2014 (Doležalová et al. 2014), two pre-emergent herbicides (Teridox 500 EC, Syngenta Czech Inc., active ingredient dimethachlor; Somero 600 SC, Arysta LifeScience Czech Inc., active ingredient pethoxamid), which showed minimal injury of rocket and should control a broad spectrum of weeds, were selected for the experiments.

Design of experiments. The experiments were conducted in the experimental fields on the periphery of the city of Olomouc (the Czech Republic, N 49°34'21.37", E 17°17'1.21", 222 m a.s.l.) in two seasons. The fields of the Crop Research Institute (the year 2015) and of the Central Institute for Supervising and Testing in Agriculture (the year 2016) were used for the experiments. The previous crops were peas with soybeans and potatoes, respectively. The first field was an example of a heavily weeded one, while the second one was an adequately weeded field. Both fields were without previous treatment with any non-selective or other herbicide. The experimental fields lie on alluvial sediments; the soils are fluvisols, with a sandy-clay or clay soil texture (Tomášek 2015). The soil pH was 6.7. During the preparation of the experimental plots, the soil was fertilized with LOVOFERT NPK 15-15-15 (Lovochemie Ltd., Czech Republic) in a dose of 50 kg/ha (i.e., 7.5 kg/ha of each of N, P_2O_5, and K_2O). Before the sowing (14th April 2015 and 20th April 2016) plots were cultivated using conventional tillage. The soil

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was sufficiently moist before the sowing and the application of herbicides. The seed volume was about 110 seeds per m^2, the sowing depth 1.5–2.0 cm, and the inter-line spacing 25 cm. The seeds were sown on plots with an area of 13.5 m^2 (width 3 m, length 4.5 m, 12 rows) using a three-row Stanhay Robin small seed drill with belt metering units (Stanhay Robin, UK).

The herbicides were tested according to the European and Mediterranean Plant Protection Organization (EPPO) standard PP 1/49 (3) for the evaluation of their efficacy in *Brassica* oil crops (OEPP/EPPO 2007). The hectare application rate recommended (RD) for rapeseed is 1 000 g/ha of dimethachlor and 1200 g/ha of pethoxamid. Four application rates (0.8 RD; RD; 1.2 RD; 2.0 RD) of each of the two selected herbicides and pure water as control were tested with the water volume 300 L/ha. The experiments were realized in four replications in randomly patterned plots.

The herbicides were applied with a HEGE 32 trial sprayer with a HEGE 76 tool carrier (Hege Maschinen GmbH, Germany) one day after the sowing of the rocket. The boom arm of the sprayer was equipped with six flat fan nozzles (Albuz API 110 02; SOLCERA Advanced Materials, France) with the height of the nozzles being 50 cm and the swath width of the spray 3 m. The application was performed at a travel speed of 2.6 km/h, a flow rate of 0.066 L/s, and a nozzle pressure of 0.2 MPa. After the application, the herbicides were not incorporated into the soil. Because of the quite low frequency and/or quantity of the rainfall during the experimental periods (see Electronic Supplementary Material – ESM), the experimental fields were irrigated as needed using above-ground sprinklers. The numbers of individuals of weed species occurring in the experimental and control plots were evaluated based on a 1m^2 area in the center of each plot at two points in time (five and seven weeks after application). The yield of rocket leaves at harvest maturity (7 weeks after application; 3 June 2015 and 8 June 2016) harvested from the 1m^2 areas was assessed. In each 1m^2 plot, the rocket plants were first counted, then harvested, and their fresh biomass was divided into consumable and inconsumable parts, which were immediately weighed.

The content of residues of the herbicides (i.e. dimethachlor and pethoxamid) was analysed at the time of the rocket maturity in the leaves harvested from the treatments with the lowest recorded phytotoxicity of the herbicides that were tested for rocket plants, i.e. for dimethachlor 0.8 RD and RD, and for pethoxamid 0.8 RD, RD, and 1.2 RD. The analyses were carried out at the National Reference Laboratory of the Central Institute for Supervising and Testing in Agriculture (Brno, Czech Republic) using the standard LC-MS method (Anonymous 2020) on an instrument UPLC-MS/MS Xevo (Waters, USA).

Climatic conditions in experimental fields. The course of the weather during the growing seasons was measured at a meteorological station located near the experimental field. The experimental periods from the sowing to the assessment and harvest lasted 51 days in 2015 and 50 in 2016. The average temperatures at the locality during those periods were 15.8 °C in 2015 and 14.5 °C in 2016, and the total precipitation was 63.4 mm in 2015 and 52.5 mm in 2016. Detailed meteorological data during the course of the experiments is given in the ESM.

Data analyses. Partial Principal Component Analysis (pPCA; Legendre and Legendre 2012) was carried out to assess the main gradients in the composition of the weed species in the plots treated with different herbicide treatments. The species data represented the logarithmically transformed number of individuals [$\log(x + 1)$]. To control for the possible effect of different seasons on the species composition, the year was used as a covariate (block) in the analysis. Each treatment group (herbicide × application rate) was visualized in an ordination diagram. Visualization of the total number of weed individuals and the number of weed taxa per plot in the ordination space was based on contour plots displaying isolines of a response surface fitted with generalized linear models (GLM). Model complexity was evaluated using the Akaike Information Criterion statistic (AIC; Šmilauer & Lepš 2014).

Constrained ordination (partial redundancy analysis, pRDA; Šmilauer & Lepš 2014) was used to test the direct effect of herbicide treatment (irrespective of herbicide type and application rate) on the species composition within the plots. The significance of the effects of herbicide treatment vs. water-treated control was tested by a Monte Carlo permutation test with 999 permutations (MC test) and the year was used as a covariate (block) in the analysis. Second constrained ordination (pRDA) was performed with identical settings to the former analysis but on a reduced dataset containing only herbicide-treated plots. The most parsimonious model was selected according to the backward elimination of insignificant effects using an MC test. To find out the weed species that were either suppressed or enhanced

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by the application of the respective herbicide or specific application rate, a *t*-value biplot was used. Because of the categorical nature of the predictors we utilized, the effect of each application rate was compared to RD. Specifically, we used Van Dobben circles, which allow easier interpretation of a t-value biplot (Lepš & Šmilauer 2014). The Canoco software (Version 5.10) (ter Braak 5.10; Šmilauer 2012) was used for the above-mentioned analyses.

The effects of the herbicide and application rate on the total number of weed plants per m^2, number of plants of most abundant weed species per m^2, and the yield of rocket (number of rocket plants, the weight of fresh consumable and inconsumable parts per m^2) were analysed separately for each year using linear models (LM) using REML estimations in NCSS (version 9). The herbicide (pethoxamid, dimethachlor) and application rate (with five levels: Control, 0.8 RD, RD, 1.2 RD, and 2.0 RD) and their interaction were considered as fixed-effect predictors. The square root of the weed density seven weeks after the application of the herbicide was considered as a covariate in the analysis of the yield of the rocket. Efficacy (in %; range 0–100%) of tested herbicide treatments on selected weeds was calculated from estimated mean weed densities, comparing results for respective application rate to control, and interpreted according to Jursík et al. (2015). Only weed species with sufficient densities (> 2 plants per 1 m^2) in the control plots were analysed for herbicide efficacy. For multiple comparisons, a Bonferroni test was used.

For the statistical analysis of the herbicide residues, nondetects data analysis (Hensel 2005) was calculated in NCSS. Specifically, the contents of residues among three treatments were compared for each herbicide. A log-rank test was used for testing the null hypotheses, that the distribution functions of two or more populations produced using the Kaplan-Meier product-limit estimator are equal. When the overall test was significant, multiple pairwise log-rank tests were calculated for each pair of treatments to find out which application rates differed from each other.

RESULTS

Effect of different application rates of two preemergent herbicides on weed species composition and density in rocket stands. Partial PCA of the composition of the weed taxa (for detailed data see ESM) differentiated the herbicide-treated and untreated control plots along the first ordination axis (capturing 38.9% of the total variation; Figure 1A). The scores of plots along the second ordination axis (8.3%) partly reflect the differences in the composition and abundance of the weed species in the control plots between the two experimental years. The control plots featured multispecies weed composition (7–17 weed taxa per 1 m^2 plot; Figure 1B and C) and dense weed populations (Figures 1D and 3) in comparison with the herbicide-treated plots. In the control plots, several annual weeds such as *Chenopodium polyspermum* Linnaeus, *Chenopodium album* Linnaeus, *Amaranthus retroflexus* Linnaeus, *Echinochloa crus-galli* (L.) P. Beauv., *Lamium purpureum* Linnaeus, *Lamium amplexicaule* Linnaeus, *Veronica persica* Poiret, and *Portulaca oleracea* Linnaeus were common and usually formed dense populations. On the other hand, the herbicide-treated plots were less diverse concerning species (less than five taxa per 1 m^2 plot) and no weed species tended to prefer herbicide-treated plots. The differences in the weed species composition between the herbicide-treated and untreated plots were significant (pRDA; MC test, pseudo$F$ = 97.6, P = 0.002, 38.3% of the adjusted explained variance).

Detailed inspection of the ordination pPCA diagram showed more extreme left positions of (*i*) the plots treated with dimethachlor than those treated with pethoxamid, and (*ii*) higher than lower application rate plots, indicating a higher efficacy of dimethachlor and higher herbicide rates in general against weeds (Figure 1A). Therefore, we further focused on a reduced dataset containing just the herbicide-treated plots. Simplification of the model resulted in a final pRDA model with just the main effects of the herbicide (*pseudoF* = 18.0, P = 0.002, 12.2% of the adjusted explained variance) and application rate (*pseudoF* = 6.4, P = 0.002, 11.4%) being significant. Dimethachlor was found to be more effective in the control of many weeds than pethoxamid (e.g. *E. crus-galli*, *Persicaria* sp., and *A. retroflexus*; Figure 2A). Comparing different application rates with the RD also showed that an application rate below the RD controlled some weeds (*E. crus-galli* and *A. retroflexus*) less efficiently than the RD (Figure 2B). 1.2 RD did not differ in its effect from the RD (Figure 2C). On the other hand, 2.0 RD controlled several weed taxa (e.g. *Lamium purpureum*, *L. amplexicaule*, *Persicaria* sp., *Thlaspi arvense* L., *A. retroflexus*, *E. crus-galli*, etc.) more efficiently than the RD (Figure 2D).

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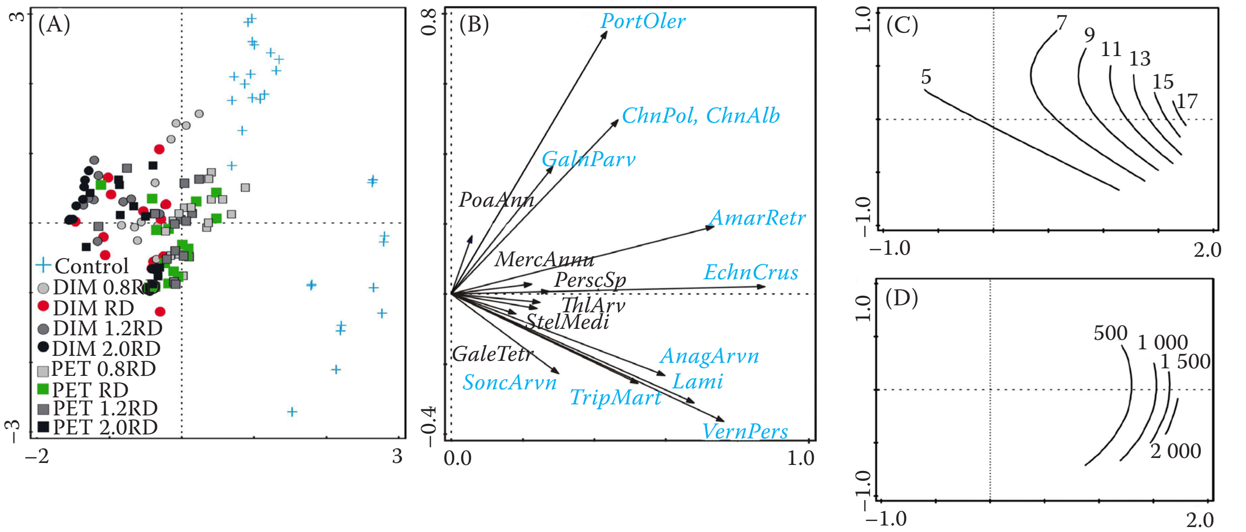

Figure 1. Partial principal component analysis (pPCA; first and second ordination axes) of weed composition in herbicide-treated and untreated control (water-treated) plots with experiments in two consecutive years treated as covariates (blocks)

(A) ordination diagram of plots with visually distinguished treatments (circles : DIM – dimethachlor; squares = PET – pethoxamid; crosses : untreated control; colouring means different doses : RD – application rate recommended for rape, 0.8, 1.2 and 2.0 are multiples of RD). (B) Ordination diagram of weed species; the taxa in blue are significantly negatively affected by any herbicide treatment, on the evidence of *t*-value biplot analysis. (C) Contour plots (GLM-based, predictors CaseR.1+CaseR.2, distribution quasi-Poisson, link function log, fitted model deviance 188.1, with 155 residual *df*s, null model deviance 311.3, with 159 residual *df*s, dispersion parameter 1.2, model *AIC* 776.3, model test $F = 25.6$, $P < 0.001$) of species richness within plots in the ordination space. (D) Contour plots (GLM-based, predictors CaseR.1+CaseR.2, distribution Gamma, link function log, fitted model deviance 204.45, with 155 residual *df*s, null model deviance 482.45, with 159 residual *df*s, model *AIC* 1631.09, model test $F = 65.1$, $P < 0.001$) of weed density within plots in the ordination space. *AmarRetr – A. retroflexus; AnagArvn – A. arvensis; ChnPol, ChnAlb* – mainly *Chenopodium polyspermum* L., sporadically *Chenopodium album* L.; *CirsArvn – Cirsium arvense* (L.) Scop.; *EchnCrus – E. crus-galli*; *EuphHeli – Euphorbia helioscopia* L.; *GaleTetr – Galeopsis tetrahit* L.; *GalnParv – Galinsonga parviflora* Cav.; *Lami – Lamium purpureum, L. amplexicaule*; *MercAnnu – Mecurialis annua* L.; *PerscSp – Persicaria* sp. div., mainly *P. maculosa*; *PoaAnn – Poa annua* L.; *PortOler – P. oleracea*; *SoncArvn – Sonchus arvensis* L.; *StelMedi – Stellaria media* (L.) Vill.; *ThlArv – T. arvense*; *TripMart – Tripleurospermum maritimum* (L.) W.D.J Koch.; *VernPers – V. persica*

The total densities of weeds seven weeks after the application of the herbicide (Figure 3) were significantly affected by the herbicide (2015: $F = 13.3$, $df = 1, 24.6$, $P < 0.001$; 2016: $F = 10.7$, $df = 1, 20.5$, $P = 0.004$) and application rate (2015: $F = 127.4$, $df = 4, 12.4$, $P < 0.001$; 2016: $F = 36.6$, $df = 4, 12.1$, $P < 0.001$), while the effect of herbicide × application rate interaction was significant in 2016 ($F = 3.6$, $df = 4, 12.1$, $P = 0.038$) but not in 2015 ($F = 0.4$, $df = 4, 12.4$, $P = 0.825$). Efficacy of dimethachlor on weeds (irrespective of concentration) was good to very good (2015: 94.9%; 2016: 95.8%), while the application of pethoxamid was slightly less effective (2015: 93.3%; 2016: 86.0%). Detailed comparisons of the effects of the application of different herbicide application rates on weed densities showed that (a) the weed densities in the untreated plots were significantly higher ($P \leq 0.05$) than the densities in every plot treated with herbicide in both years (Figure 3A, B); (b) despite a trend of decreasing weed densities with an increasing herbicide application rate, only 0.8 RD and RD resulted in statistically less effective suppression of weed densities compared to the plots treated with 2.0 RD in 2015 (Figure 3A); (c) except for 1.2 RD, pethoxamid and dimethachlor did not differ statistically in their effects on the weed density in 2016 (Figure 3B).

The efficacy of the tested herbicides on weeds was studied in detail for the eight most abundant taxa (Table 1). Dimethachlor fully controlled *Echinochloa crus-galii*, *Veronica persica*, and *Amaranthus retroflexus* (in 2016) from the lowest application rate. Control of *Amaranthus retroflexus* (in 2015) and *Portulaca oleracea* (90–99% and 91–97%, respectively) was good to very good. Only the highest tested application rate controlled *Thlaspi arvense* acceptably. *Chenopodium polyspermum* was controlled effectively only by the two highest application

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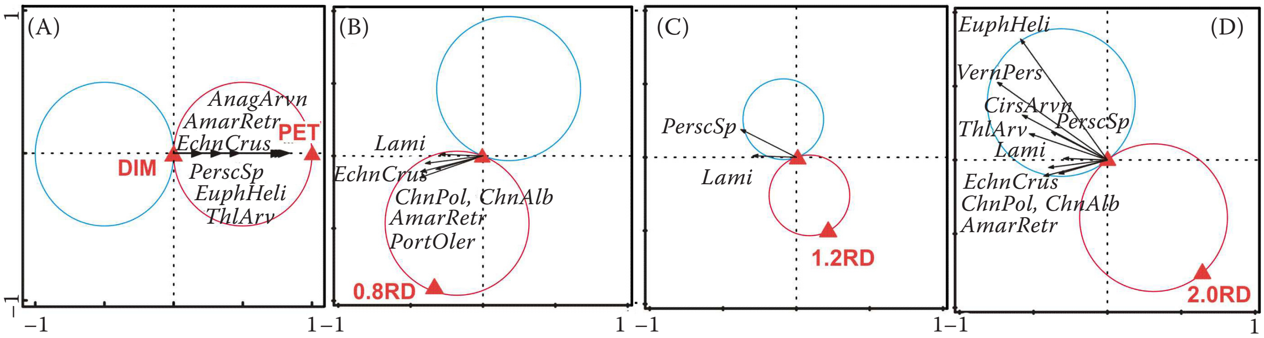

Figure 2. The t-value biplots and Van Dobben circles that summarize the t-statistics of the regression coefficients representing the relation of each weed species in a constrained ordination (pRDA) to the predictors (herbicide, application rate)

(A) effect of pethoxamid (PET) compared with dimethachlor (DIM) as predictors, irrespective of application rate; (B–D) Effect of each application rate (0.8 RD, 1.2 RD, 2.0 RD as predictors) compared to a RD (triangle in the centre of the diagrams), irrespective of the herbicide used. Van Dobben circles were plotted in pairs reflecting positive (coloured red) or negative effects (coloured blue) of selected predictor variables on weed species. When a species' arrowhead ends within a positive response circle (plotted in red), the effect of the predictor variable upon that response variable is judged significant, because the t-value statistic from a (multiple) regression is predicted to have a value greater than two. When the arrowhead falls within the negative (blue) response circle, a significant negative response (based on a t-value less than –2) is predicted (ter Braak & Šmilauer 2012). Only the weed species significantly affected by the respective predictor are reported in figures. For species abbreviations see Figure 1

rates under low weed infestation (2016). Dimethachlor controlled *C. polyspermum* insufficiently under intensive weed infestation (2015). Control of *Persicaria* sp. was insufficient.

The highest tested application rate of pethoxamid fully controlled *Portulaca oleracea*, *Veronica persica*, *Amaranthus retroflexus*, and *Echinochloa crus-galii*. The efficacy of other application rates on the mentioned species was good to very good. Only the highest application rate acceptably controlled *Chenopodium polyspermum*. Control of *Thlapsi arvense* and *Persicaria* sp. was insufficient. Efficacy of both tested herbicides was very good (96–100%) on *Lamium purpureum* and *L. amplexicaule*, even from the lowest tested application rate. Both herbicides fully controlled *Lamium* with 2.0 RD.

Effect of different application rates of two pre-emergent herbicides on yield parameters of rocket stands. The analysis of the production traits of the rocket showed marked differences between the

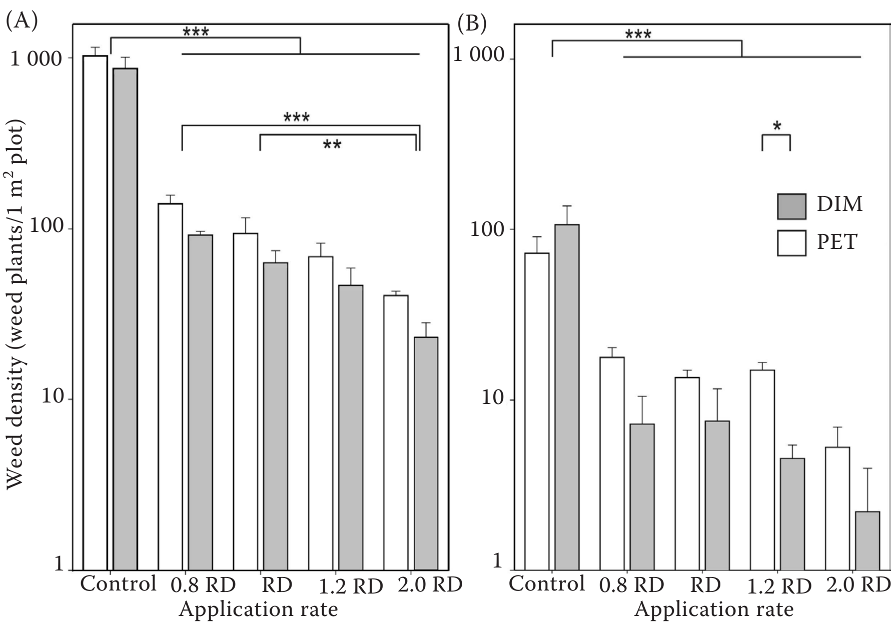

Figure 3. Weed densities (mean + SE; plants per 1 m^2) in the untreated control plots and plots treated with different application rates (0.8, 1.0, 1.2 and 2.0 times the recommended application rate, RD) of two herbicides (dimethachlor (DIM) and pethoxamid (PET)) in (A) 2015 and (B) 2016

Note the log-scale of weed density. Significant differences between groups (Bonferroni test) are marked (* $0.01 < P \leq 0.05$, ** $0.001 < P \leq 0.01$, *** $P \leq 0.001$). For details, including the results of LM, see the text

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Table 1. Efficacy (in %) of the tested application rates (0.8, 1.0, 1.2, and 2.0 of RD) of two herbicides (dimethachlor, pethoxamid) on selected weed species shortly before the rocket harvest (seven weeks after sowing)

Herbicide	App. rate	*Lamium purpureum, L. amplexicaule*	*Persicaria* spp., mainly *P. maculosa*	*Echinochloa crus-galli*		*Chenopodium* spp., mainly *C. polyspermum*		*Thlaspi arvense*	*Amaranthus retroflexus*		*Veronica persica*	*Portulaca oleracea*
		2015	2015	2015	2016	2015	2016	2015	2015	2016	2015	2016
Dimethachlor	0.8	97^{a}	45	99^{a}	100^{a}	0^{a}	63^{a}	43^{a}	90^{a}	100^{a}	100^{a}	91^{a}
Dimethachlor	1.0	98^{a}	55	100^{b}	100^{a}	36^{a}	74^{a}	50^{a}	97^{a}	100^{a}	100^{a}	97^{a}
Dimethachlor	1.2	99^{a}	51	100^{b}	100^{a}	0^{a}	100^{a}	79^{ab}	97^{a}	100^{a}	100^{a}	97^{a}
Dimethachlor	2.0	100^{b}	69	100^{b}	100^{a}	69^{a}	96^{a}	90^{b}	99^{a}	100^{a}	100^{a}	100^{a}
Pethoxamid	0.8	96^{a}	0	94^{a}	73^{b}	17^{b}	56^{a}	51^{a}	89^{a}	90^{a}	97^{a}	96^{a}
Pethoxamid	1.0	97^{a}	27	97^{a}	91^{ab}	69^{b}	81^{a}	50^{a}	89^{a}	100^{a}	98^{a}	100^{a}
Pethoxamid	1.2	98^{a}	52	98^{a}	91^{ab}	74^{b}	81^{a}	48^{a}	85^{a}	97^{a}	98^{a}	99^{a}
Pethoxamid	2.0	100^{b}	67	99^{ab}	100^{a}	92^{b}	89^{a}	70^{ab}	97^{a}	100^{a}	100^{a}	100^{a}
Weed density in control plots (ind./m^2)		169 (90)	32 (33)	568 (141)	20 (12)	13 (16)	7 (4)	51 (38)	66 (41)	10 (5)	56 (41)	52 (54)
Herbicide		1.6 (0.221)	0.4 (0.520)	85.4 (< 0.001)	28.1 (< 0.001)	7.0 (0.014)	2.6 (0.119)	1.6 (0.221)	1.6 (0.229)	3.2 (0.085)	0.5 (0.511)	4.4 (0.051)
Application rate		64.6 (< 0.001)	2.0 (0.160)	259.5 (< 0.001)	43.7 (< 0.001)	3.0 (0.063)	15.8 (0.001)	8.3 (0.022)	7.7 (0.002)	62.2 (< 0.001)	68.2 (< 0.001)	42.4 (< 0.001)
Herbicide × application rate		0.5 (0.737)	0.7 (0.640)	12.7 (< 0.001)	2.3 (0.083)	0.1 (0.980)	0.9 (0.513)	3.5 (0.043)	1.2 (0.370)	0.8 (0.510)	0.7 (0.592)	1.1 (0.393)

The effects of herbicide, application rate and their interaction on weed density (mean and SD) were tested by the LM; the test value (F) and respective P-value in parentheses are reported for each test; efficacy values within a column with the same letter are not significantly different at $P \leq 0.05$ (Bonferroni test); RD – application rate recommended for a rapeseed is 1000 g/ha of dimethachlor and 1200 g/ha of pethoxamid; four application rates are multiplies of RD (0.8 RD; RD; 1.2 RD; 2.0 RD)

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experimental years. In 2015, rocket plants were pronouncedly smaller and their leaves were yellowish or slightly reddish in the control plots, while the plants were much larger and with bigger leaves in the herbicide-treated plots. No significant effects of weed density, herbicide, application rate, and their interaction on the number of rocket plants and the weight of consumable parts per m^2 were found (Table 2). Only one effect was found significant in the case of inconsumable parts: the application of dimethachlor significantly reduced the weight of inconsumable parts in comparison with the application of pethoxamid. The data also showed a marginally significant trend: the application of herbicide in any application rate increased the weight of consumable rocket parts in comparison with the untreated control (contrast control vs. the average of four application rates: F = 4.38, P = 0.072; Figure 4).

In 2016, the application of dimethachlor in any used application rate caused a strong reduction in the density of the rocket (even more drastic in the case of 2.0 RD), while only the effect of the 2.0 RD of pethoxamid reduced the density of the rocket significantly. The weed density had no significant effect on the rocket yield at all. The application of 0.8 RD, RD, and 1.2 RD of dimethachlor, significantly reduced the weights of both the consumable and inconsumable parts of the rocket. The application of 2.0 RD of pethoxamid caused a drop in the weight of inconsumable parts in comparison with other tested application rates of pethoxamid. The most extreme drop in the weights of both consumable and inconsumable parts of the rocket was caused by the application of 2.0 RD of dimethachlor (Table 2, Figure 4).

Herbicide residue assessment. The content of residues of the herbicides was analysed in the leaves at the time of the rocket maturity. Concerning dimethachlor, all measurements fell below the detection limit (0.003 mg/kg). On the other hand (overall log-rank test, χ^2 = 10.3, df = 2, P = 0.006), rocket leaves sampled in plots treated with the RD of pethoxamid had significantly higher concentra-

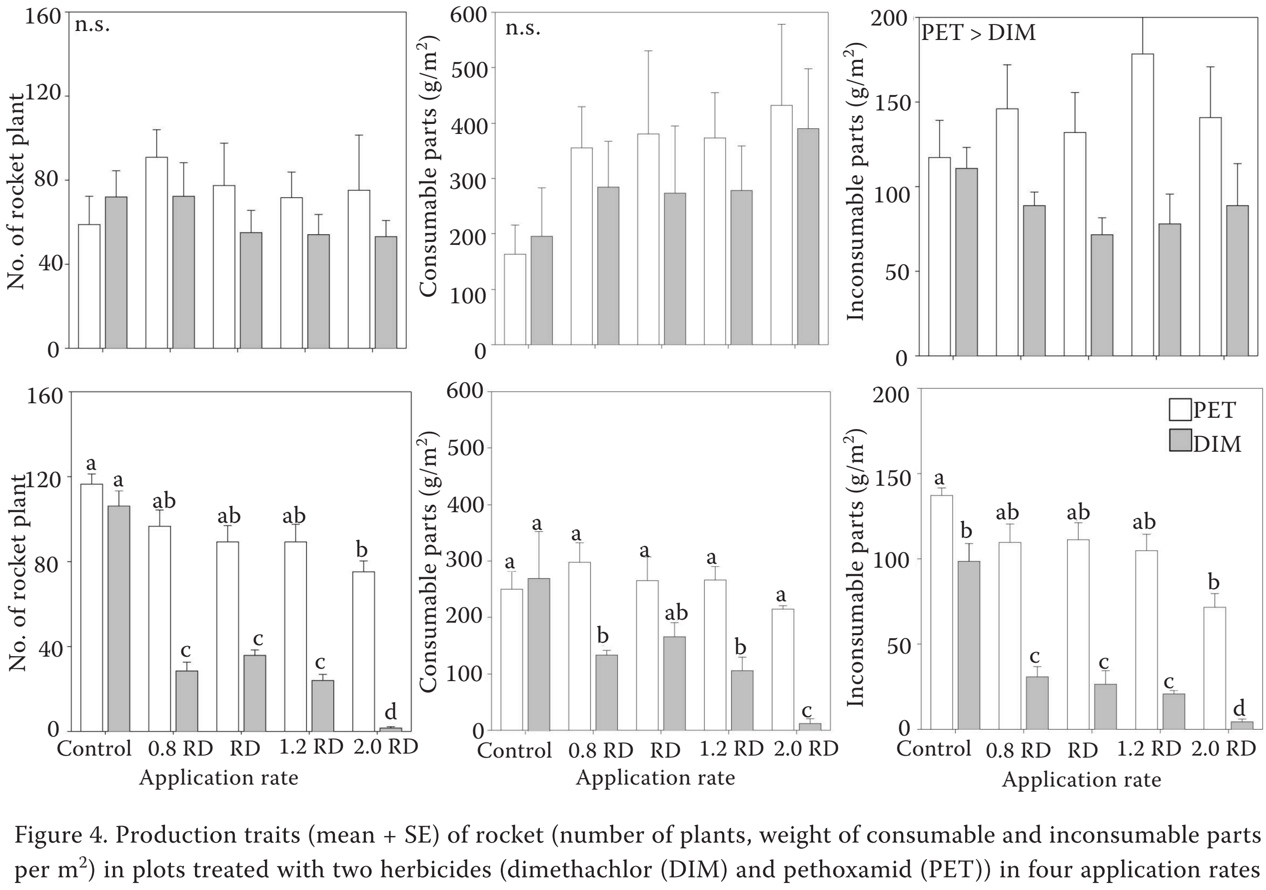

Figure 4. Production traits (mean + SE) of rocket (number of plants, weight of consumable and inconsumable parts per m^2) in plots treated with two herbicides (dimethachlor (DIM) and pethoxamid (PET)) in four application rates (0.8 RD, RD, 1.2 RD and 2.0 RD) in comparison with the untreated control (Control)

Upper row: results of 2015 experiment; lower row: results of 2016 experiment; significant differences between groups (Bonferroni test at $P \leq 0.05$) are marked by different letters, separately for each trait and year; n.s – no significant difference; PET > DIM – effect of pethoxamid greater than effect of dimethachlor at $P \leq 0.05$

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Table 2. Effect of weed density, herbicide, application rate and their interaction on the yield parameters of rocket in the two experimental years (2015, 2016) analyzed using linear models

Term	2015				2016			
	F	df_G	df_E	P	F	df_G	df_E	P
Number of rocket plants per 1 m² $								
Weed density$	0.37	1	12.9	0.552	2.69	1	8.8	0.136
Application rate	0.54	4	13.9	0.709	9.63	4	13.1	< 0.001
Herbicide	0.59	1	26.3	0.449	261.53	1	28.4	< 0.001
Application rate × herbicide	0.44	4	12.0	0.775	14.67	4	12.0	< 0.001
Weight of consumable parts (g/m²)$$								
Weed density$	0.07	1	17.9	0.802	0.00	1	13.3	0.99
Application rate	0.17	4	14.7	0.952	4.62	4	11.7	0.017
Herbicide	0.28	1	23.4	0.604	45.25	1	11.73	< 0.001
Application rate × herbicide	0.14	4	12.1	0.963	4.65	4	11.0	0.019
Weight of inconsumable parts (g/m²)$$								
Weed density$	2.05	1	13.2	0.176	2.26	1	6.8	0.177
Application rate	0.77	4	13.6	0.563	7.04	4	11.8	0.004
Herbicide	6.24	1	22.2	0.020	172.77	1	20.1	< 0.001
Application rate × herbicide	1.14	4	11.2	0.385	10.15	4	11.7	0.001

$ square root transformed; $$ log(x + 1) transformed

tions of herbicide (median and its 95% confidence interval: 0.003, 0.002–0.007 mg/kg) than those treated with 0.8 RD (χ^2 = 6.0, df = 1, P = 0.015) or untreated with pethoxamid (χ^2 = 4.5, df = 1, P = 0.034), where all the measurements fell below the detection limit (comparison Control-0.8 RD: χ^2 = 0.0, df = 1, P = 1.000). However, the contents of the residues of both herbicides in the rocket leaves treated with different application rates of herbicide were all below the 'default limit' of 0.01 mg/kg.

DISCUSSION

In both experimental years, clear effects of both tested herbicides against weedy species were recorded compared to the untreated controls (Figure 1A). Higher application rates of herbicides were more effective than lower ones (Figure 1A, 2A and 3). Dimethachlor showed better weed control (efficacy on total weed spectrum 94.9–95.8%). Excellent efficacy (good to full control) of dimethachlor from the lowest tested application rates was recorded for *Echinochloa crus-galli*, *Portulaca oleracea*, and *Amaranthus retroflexus* (Table 1). Results are in accordance with Wan et al. (1992) who reported 100% efficacy against *Echinochloa crus-galli* and 60–100% control of *Portulaca oleracea* and *Amaranthus tricolor* at the application rate 1119 g/ha. The excellent efficacy of dimethachlor from the lowest application rate was also observed on *Lamium purpureum* (very good to full control) that is in line with PPDB (2020) and *Veronica persica* (full control). This herbicide is perhaps also effective against *Galinsoga parviflora*, because it was recorded in the untreated plots but not in the adjacent experimental plots. But we cannot confirm this as we do not know whether it was due to the efficacy of the herbicide or the real absence of the species from the treated plots. Low to acceptable control was also recorded against *Thlaspi arvense* and *Cirsium arvense*. Our experiments did not show efficacy against *Persicaria* sp. div. and *Chenopodium* sp. div. Dimethachlor should be efficient also against *Alopecurus myosuroides* Huds., *Apera spica-venti* (L.) P. Beauv., *Bromus* L., *Poa* L., *Stellaria media* (L.) Vill. and *Tripleurospermum* Sch. Bip. (PPDB 2020).

Pethoxamid was less effective against the monitored weed species in comparison with the effect of dimethachlor. However, in comparison to the untreated plots, the reduction in the total numbers of weed plants was also pronounced (86.0–93.3%), which could be considered as an acceptable to good weed control on fields with lower weed infestation. Nevertheless, there were mostly no obvious differences in the numbers of surviving weed plants when the plots treated with different herbicide application rates were compared, except for higher efficacy of 2.0 RD. Pethoxamid showed excellent efficacy (mostly good to very good control) on *E. crus-galli* that is in line with Anonymous (2002), Hunt et al. (2015), Jursík et al. (2015; efficacy 90–97%), and

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PPDB (2020). Pethoxamid also very well controlled *Lamium purpureum*, *L. amplexicaule*, and *Veronica persica.* In contrast to the insufficient efficacy of pethoxamid on *Portulaca oleracea* reported by Anonymous (2002), we found very good to full control of this species by this herbicide (Table 1). Using higher application rates (≥ RD), pethoxamid sufficiently or acceptably controlled *Anagallis arvensis* and *Chenopodium* spp. Anonymous (2002) and PPDB (2020) reported the efficacy of pethoxamid on *Chenopodium album*, however, Jursík et al. (2015) found insufficient control. Our experiments did not confirm the efficacy of pethoxamid on *Persicaria maculosa* reported by PPDB (2020), although also according to Anonymous (2002) the control of pethoxamid should be insufficient on this species. We also found the efficacy of pethoxamid on *A. retroflexus* (Table 1) comparable with previous research (Anonymous 2002; Hunt et al. 2015; Jursík et al. 2015: 91–99%; Soltani et al. 2019: 98%). Control of *Thlaspi arvense* was low to insufficient. According to available data, pethoxamid should be efficient also on *Convolvulus arvensis* Linnaeus, *Digitaria* Haller, *Panicum* Linnaeus, *Setaria* P. Beauv., and *Solanum* Linnaeus (Hunt et al. 2015; PPDB 2020).

Our results showed different effects of the two herbicides on the rocket yield parameters, depending on the year of the experiment. Such contrasting results emphasize the importance of repeated testing under contrasting environmental conditions. Dimethachlor showed lower selectivity to a rocket than pethoxamid and it, therefore, reduces the production of the rocket. In 2015, the experiment was carried out in a heavily weeded field, which proved to be an advantage, as both herbicides could be tested on the efficacy on a broad spectrum of weed species and on their density. Despite the significant effect of the herbicides against the weeds, only 50–75% of the initially sowed rocket seeds reached maturity, irrespective of the treatment. One possible explanation of such a contrasting result could be that the high densities of weeds caused a strong competitive effect early in the season (Gallandt & Weiner 2015; Pannacci & Onofri 2016) that strongly affected the growth of the rocket seedlings. However, a marginally significant trend of the weight of consumable rocket parts increasing after the application of any tested doses of herbicides was recorded in the 2015 experiment, confirming the positive effect of herbicide treatment on the final consumable yield of the rocket as a result of the removal of competition from weeds. Growth reduction and a significantly lower yield of crops in untreated plots as a result of competition from weeds have been reported by many authors (e.g. Ritter et al. 2008; Pannacci & Onofri 2016). The application of dimethachlor, however, caused poorer development of inconsumable rocket parts in comparison with the application of pethoxamid.

In 2016, we found high rocket germination and survival approaching 90–100% in untreated plots and a low (pethoxamid) or high decrease (dimethachlor) in rocket densities in the herbicide-treated plots. Because the weed densities were ten times lower in comparison with the 2015 experiment when an effect of "dilution" of applied doses of herbicides among extremely high numbers of weed individuals could occur, it is likely that in 2016 a higher proportion of the applied dose of herbicide left in the soil and could, therefore, have been absorbed by the growing rocket plants, leading either to neutral (pethoxamid) or even disadvantageous (dimethachlor) effects on the germination and development of the rocket. To summarize, any herbicide treatment tended to increase the yield but also the variation in the consumable rocket parts in the 2015 experiment, suggesting a weak positive effect of the herbicide treatment on the rocket yield, which is generally expected in strongly weeded trials when a threshold weed density level for crop stands is exceeded (Ritter et al. 2008; Gerhards et al. 2011). However, if the weed competition is relatively low, as observed in the year 2016, the total effect of the herbicide treatment on the yield is usually close to zero or negative, as in our case, as a result of its phytotoxic effect (Gerhards et al. 2011).

Considering the usability of the herbicides in the rocket, pethoxamid seems to be more suitable, despite its slightly lower efficacy on weeds. It follows that the survival of the rocket plants and the yield of consumable parts were higher than, or comparable to, the control plots after the application of pethoxamid in any application rate that was applied, compared to the mostly negative effects of dimethachlor on the rocket yield. Moreover, even the lowest herbicide application rate used in the experiments (0.8 RD) is sufficient to satisfy both the rocket yield and weed control. Though the RD of both herbicides was recommended for oilseed rape and not for a rocket, our data is in agreement with other studies on different crops that demonstrate the overestimation of recommended doses for many herbicides (Zhang et al. 2000; Gaba et al. 2016). Herbicides in reduced doses are

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often sufficient to control weed density at or below the threshold levels and thus maintain satisfactory crop yields (Steckel et al. 1990; Gaba et al. 2016).

In food, herbicide residues are found minimally, compared to insecticides or fungicides (Anonymous 2017; EFSA 2018). However, evaluation of which herbicide and in which application rate is suitable for use in a certain crop in terms of harmlessness to health at the stage of harvest maturity must be based on analysis of the residues of herbicides in the resulting product (Keikotlhaile & Spanoghe 2011). The RDs used in our study were primarily based on the application doses recommended mainly for rapeseed (Anonymous 2014), which is harvested for seed and therefore has a much longer time from the application of the herbicide to harvesting to degrade the residues. In the rocket, the dimethachlor residue was below the limit of quantification (LOQ; 0.003 mg/kg) when the RD was applied. This finding is consistent with the results of Šuk et al. (2018), which reported fast degradation of dimethachlor and recommended it for non-residue production and for products intended as infant food. The toxicological evaluation and environmental behaviour studies allow the classification of pethoxamid into the safe herbicide group (Kato et al. 2001). Rocket harvested from the plots treated with pethoxamid using the RD had weakly positive findings but all below 0.010 mg/kg (a 'default limit'), which is the baseline MRL for food, unless otherwise specified (Anonymous 2005). However, since the pethoxamid residue content in the leaves harvested from the 0.8 RD-treated plots did not differ significantly from the untreated plots in either of the experimental years and the pethoxamid content was always below the limit of quantification, we have decided to recommend this dose, i.e., 960 g/ha of pethoxamid, for weed control in rocket stands.

CONCLUSION

Pethoxamid showed no or minimal phytotoxicity to the rocket. Although this herbicide is slightly less effective against weeds than dimethachlor, pethoxamid eliminates many weeds reliably. The dose 960 g/ha is safe in terms of the presence of residues in the rocket leaves at harvest maturity. The time from the pre-emergent application of the herbicide to the harvesting of the rocket leaves, which is about seven weeks, is sufficient for the degradation of pethoxamid in the harvested product.

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