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## DIFFERENCES BETWEEN SOIL-APPLIED HERBICIDE TREATMENTS IN CONTROL OF SPRING AND SUMMER-GERMINATING ANNUALS IN TOBACCO (*Nicotiana tabacum* L.)

### SUMMARY

The field trials were conducted during two tobacco growing seasons (2020 and 2021) to evaluate differences between soil-applied herbicide treatments in control of spring and summer-germinating annuals in tobacco. *Amaranthus retroflexus* L. (AMARE), *Portulaca oleraceae* L (POROL), *Chenopodium album* L. (CHEAL), *Polygonum aviculare* L. (POLAV), and *Digitaria sanguinalis* L. (DIGSA) were the most dominant weeds in both regions. Overall performances of the PRE-T and POST-T (OT) herbicide treatments were not due to inconsistent weather conditions between the 2 years of the study only, but to working way of tobacco seedling transplanting machine, as well. Higher amount of precipitation during the 10 days after (DA) PRE-T herbicides application caused PRE-T herbicide leaching which lowered efficacy of PRE-T herbicides in control of predominant weeds (<76%) compared to their application as POST-T (OT) treatment (>91%) in 2020 in Prilep region. Precipitation above 30ys average were recorded in Titov Veles, as well, but due to their equal occurrence, as well as soil type characteristics, leaching did not occur and efficacy was good for PRE-T (77-87%) and excellent for POST-T (OT) (95-100%) treatment. Rainfalls 10 DA after POST-T (OT) application in 2021 were above the 30ys average for Prilep region, which caused significant leaching of herbicides and reduction of POST-T (OT) herbicide efficacy (<74) compared to PRE-T treatment (76-88%). Opposite, dry condition after PRE-T application and POST-T (OT) application as well, may have contributed to the poor performance of both PRE-T and POST-T (OT) herbicide treatments (<76% and <79%, respectively) in Titov Veles region in 2021. Heavy precipitation directly following PRE-T and POST-T (OT) application caused tobacco injury in Prilep region in 2020 and 2021, which ranged from 10 to 30% and 12 to 27%, respectively across both treatments 7 DAHA. Injures of pendimethalin and metolachlor were more serious. Tobacco

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yields for each treatment in both regions generally reflected overall weed control and crop injury.

**Keywords:** efficacy, weeds, herbicide treatment

## INTRODUCTION

Quality and quantity of tobacco depend on the implementation of good agriculture practices (GAP), including, good management of weeds (Stocks, 1994). Tobacco is one of the most vulnerable crops to weed interference. The losses in crop yield are not only due to weed competition effects, but to the fact that weeds serve as host for various pest and diseases harmful to the crop (Rao, 2000). Further, they reduce leaf yield and quality, interfere with mechanical harvest, contribute to foreign matter contamination in marketable leaf, and can compromise export market opportunities (Lolas, 1986; Bailey, 2013). Many of the common weed problems in tobacco are spring and summer-germinating annuals (Vouzounis and Americanos, 1995; Travlos *et al.* 2014; Bailey *et al.* 2014). Most of them, such as *Polygonum aviculare* L. (POLAV), *Amaranthus retroflexus* L. (AMARE), *Portulaca olearacea* L. (POROL), *Chenopodium album* L. (CHEAL), and *Digitaria sanguinalis* (L.) Scop (DIGSA) are problematic in tobacco production regions in N. Macedonia (Pacanoski and Glatkova, 2010). POLAV is a widespread weed of agricultural systems affecting many crops (Lemerle *et al.* 1996; Lemerle *et al.* 2001; Amor and Francisco, 1987). Irrespective of wheat density, there was a 19% reduction in wheat dry matter between one and three POLAV plants per pot, and a 57% reduction in wheat dry matter between three and six POLAV plants per pot (Burnett *et al.* 2008).

The POLAV caused an average chickpea seed yield loss of 14, 46, 74 and 88% at the density of 4, 8, 16, 32 plants m<sup>-2</sup> compared to the weed-free crop (Radicetti *et al.* 2012). POLAV is one of the most widespread weed species in summer crops (Gonnella *et al.* 2010). Seedling lettuce is especially vulnerable to POLAV competition (Lanini and LeStrange, 1991). In Florida's lettuce fields, season-long interference by this species caused yield reductions of 48% (Santos *et al.* 1997). AMARE is listed as one of the most common dicotyledonous weeds in the world and is widely distributed in many agricultural areas (Horak and Loughin, 2000).

The presence of AMARE can severely reduce the yield of several crops, such as beans (Mirshekari *et al.* 2010; Amini *et al.* 2014), soybean (Bensch *et al.* 2003), maize (Sheibany *et al.* 2009; Ghanizadeh *et al.* 2014), and cotton (Buchanan *et al.* 1980). The maize yield loss due to AMARE competition was reported to be from 5–34% (Kenzevic *et al.* 1994) and ≤50% (Bosnic and Swanton, 1997), depending on the *its* density. When two AMARE plants m<sup>-1</sup> per row emerged with soybean, the crop yield was reduced by 12.3% (Dielman *et al.* 1995). The full-season competition of 120 AMARE plants m<sup>-2</sup> with snap bean reduced the crop yield by 75% (Aguyoh and Masiunas, 2003a). CHEAL is one of the most competitive annual broadleaf weeds in agricultural systems especially in

maize and soybean (Boerboom, 2009; Kruger *et al.* 2009). Conley *et al.* (2003) reported a 61% reduction in soybean yield due to season-long interference from CHEAL densities of 64 plants m<sup>-2</sup>.

Sibuga and Bandeen (1980) found that maize yield was reduced by as much as 58% when CHEAL density reached 277 plants m<sup>-2</sup>. In vegetable crops, season-long interference by CHEAL has resulted in 36 and 65% yield reductions in tomato and lettuce, with 64 and 133 plants m<sup>-1</sup> of row, respectively (Bhowmik and Reddy, 1988; Santos *et al.* 1997). DIGSA is a serious problem in many row crops particularly common in maize, beet, vegetable crops, orchards and vineyards (Bhowmik *et al.* 1999; Sarker *et al.* 2002; Fu and Ashley, 2006). After competition from DIGSA for 6 weeks and for a full season, snap bean yields were reduced by 28 and 72%, respectively, and leaf area was reduced by 40 and 48%, respectively (Lugo and Talbert, 1989). Early-emerging DIGSA reduced snap bean biomass 10 to 28% and snap bean pod numbers 44 to 60%, depending on the density (0.5 to 8 plants m<sup>-1</sup>) (Aguyoh and Masiunas, 2003b).

With respect to weed control, this weed flora has been traditionally controlled with soil applied herbicides, due to a scarce availability of POST-em herbicides. A few soil applied herbicides are currently registered for use in tobacco in R.N. Macedonia. Pendimethalin is a dinitroaniline selective herbicide that controls mainly annual grasses (*Echinochloa crus-galli*, *Setaria* spp., *D. sanguinalis*), and some annual broadleaf weeds (*A. retroflexus*, *C. album*) in many crops (Hatzinikolaou *et al.* 2004; Lin *et al.* 2007; Soltani *et al.* 2015). Metobromuron belongs to the class of substituted ureas and is usually applied to control annual broadleaf weeds such as *C. album*, *A. retroflexus*, *Polygonum* spp. in sunflower, soybean, and common bean crop. Metobromuron controls certain annual grasses including *Setaria* spp (Vischetti *et al.* 2002).

Aclonifen, a diphenyl ether herbicide, has the capability to inhibit protoporphyrinogen oxidase in a manner that is dependent on light exposure (Choi *et al.* 1999; Grasset *et al.* 2011). It is used frequently in various crops against broadleaved (*Ch. album*, *A. retroflexus*) and some grass weeds (*E. crus-galli*, *Setaria* spp.) (Kilinc *et al.* 2009). S-metolachlor and dimethenamid, both chloroacetamide herbicides, control annual grasses *Setaria* spp., *D. sanguinalis*, *E. crus-galli*, *Cyperus esculentus*, and some small-seeded broadleaf weeds, including *Amaranthus* spp. and *Solanum* spp. (Moseley and Hagood, 1990; Osborne *et al.* 1995; Clewis *et al.* 2001; Vann *et al.* 2019; Clapp *et al.* 2020).

Taking into account that these herbicides are usually applied PRE-T (pre transplantation), but never POST-T (OT) (post transplantation over-the-top) of tobacco (within 5 days of transplanting), the objectives of the present study were to evaluate differences between PRE-T and POST-T (OT) herbicide treatments in control of spring and summer-germinating annuals, and to estimate their influence on tobacco yield and injury effect on tobacco crop, as well.

## MATERIAL AND METHODS

### Study site description

The field trials were conducted during two tobacco growing seasons in 2020 and 2021 on commercial tobacco fields in the Prilep (locality Galičani - 41°18'18"N 21°28'41"E) and Titov Veles (locality Stari Grad - 41°37'N 21°42'E) tobacco growing regions in south-western and central N. Macedonia. The soil at Prilep region was a fluvisol sandy loam with 18.50% coarse, 55.10% fine sand, 26.40% clay + silt, 1.56% organic matter and pH of 6.9. The soil at Titov Veles region was a vertisol with 3.50% coarse, 34.20% sand, 60.30% silt + clay, 2.4% organic matter and pH of 7.2 (Filipovski, 2006).

### Experimental design and treatments

The tobacco was grown following conventional tillage practices. The soil was prepared by moldboard plowing in the autumn followed by two passes with a field cultivator in the spring. Before transplanting in the spring, fertilizer was incorporated at rates indicated by soil tests.

**Table 1.** List of treatments, herbicide active ingredient commercial products, herbicide rates, and producers used in field experiments to evaluate differences between PRE-T and POST-T (OT) treatments in control of spring and summer-germinating annuals in tobacco (*Nicotiana tabacum* L.) at Prilep and Titov Veles, NMK, in 2020 and 2021

Treatments	Content of a.i.	Trade name	Rate (L/ha)	Supplier
Untreated control	-----	-----	-----	-----
Weed-free control	-----	-----	-----	-----
Pendimethalin	455 g/L	Stomp Aqua	3.0	BASF Agro B.V, Switzerland
Metobromuron	500 g/L	Proman	3.0	Belchim Crop Protection, Belgium
Clomazone	480 g/L	Gamit 4F	1.0	FMC Agro Limited, UK
Aclonifen	600 g/L	Challenge 600 EC	4.0	Bayer Crop Science, Germany
S-Metolachlor	960 g/L	Dual Gold 960	1.5	Syngenta International, Switzerland
Dimethenamid	900 g/L	Frontier 900 EC	1.7	BASF, Germany

The field studies were carried out with tobacco type "Prilep" cv. P-23 which was transplanted in a well-prepared soil on May 26<sup>th</sup>, 2020 and May 19<sup>th</sup>, 2021 in the Prilep region, and on May 18<sup>th</sup> 2020 and May 11<sup>th</sup> 2021 in Titov Veles region, respectively. The interrow/inrow plant spacing was 40 x 15 cm (166.667 plants/ha). The trials were conducted in two different sites of the same commercial tobacco fields. Herbicides were applied with a CO<sub>2</sub>-pressurized backpack sprayer calibrated to deliver 300 L/ha aqueous solution at 220 kPa. Application of herbicides was made 2 days PRE-T and 5 days POST-T (OT). At

the time of treatment, the weeds were in the dry seed to the beginning of seed imbibition growth stage (BBCH 00-01). The design of the experiment was structured as a randomized complete block system that included four replicates, and the size of the harvest plot was 20 m<sup>2</sup>. The following treatments were included in the study (Table 1).

Untreated and weed-free controls were included in the studies, as well. The weedy control plots were left untreated during the entire experimental period. Weed-free control was maintained by hand weeding. Hand-weeding was initiated at weeds emergence and continued as needed to maintain weed-free plots.

### Data Collection

Weed control efficacy was estimated 28 days after herbicides application (DAHA) after weed emergence (6-8 tobacco leaves, BBCH 16-18) by the weed plants counting from 1m<sup>2</sup> area within each plot, and herbicide efficacy was calculated by equitation (Chinnusamy *et al.* 2013):

$$WCE = \frac{W_{up} - W_{tp}}{W_{up}} * 100$$

where:

W<sub>CE</sub> - weed control efficiency

W<sub>up</sub>- number of weeds in the untreated plots

W<sub>tp</sub>- number of weeds in the treated plots

Tobacco injury was visually evaluated based on a 0% - 100% rating scale, where 0 is no injury to tobacco plants and 100 is complete death of tobacco plants (Frans *et al.* 1986). Visual estimates of percent tobacco injury were estimated 7 and 21 DAHA, by determining the average percentage of deformation, plant stunting, chlorosis, or necrosis (or all) occurring in treated tobacco plant when compared with nontreated plants at both localities during two-year experimental period. Yields were taken by the mechanically harvesting from every plot. Individual plots were harvested a total of six times each year as they matured and ripened to achieve optimum leaf quality and grade. Harvest started on July 9<sup>th</sup>, 2020 and July 1<sup>st</sup>, 2021, in the Prilep region, and on July 2<sup>nd</sup>, 2020 and June 26<sup>th</sup>, 2021 respectively, and was harvested every 13 to 15 day thereafter, depending on the year.

### Meteorological conditions during and after the herbicide application

Total rainfall, as well as average temperatures 10 DA PRE-T and 10 POST-T (OT) applications, respectively were recorded (Tab. 2). In 2020 the first few DA PRE-T application in Prilep region were unusually wet, particularly the 3<sup>rd</sup>, 4<sup>th</sup>, and 5<sup>th</sup> day (15, 11 and 10 mm, respectively). Rainfall occurred after POST-T (OT) application was in the line with the average for Prilep region. In Titov Veles region for the same year rainfall occurred in the 1<sup>st</sup> decade of PRE-T application were little above the 30ys average for this region. A little bit above the 30ys average for this region were precipitations occurred in the 1<sup>st</sup> decade of POST-T (OT) application. In 2021, in Prilep region precipitation occurred 10 DA

PRE-T application were in the line with the average for this region, while precipitation occurred POST-T (OT) application were for 22% above the 30y average for this region. In Titov Veles region, in the same year, period during the 1<sup>st</sup> decade of PRE-T and POST-T (OT) application was very dry (in total for 10 days only 6 and 11 mm, respectively) (Tab. 2).

**Table 2.** Rainfall and temperature data 10 DA PRE-T and 10 POST-T (OT) applications, respectively at Prilep and Titov Veles region in 2020 and 2021<sup>a</sup>

Date	Prilep region								Date	Titov Veles region							
	2020				2021					2020				2021			
	PRE-T		POST-T (OT)		PRE-T		POST-T (OT)			PRE-T		POST-T (OT)		PRE-T		POST-T (OT)	
	P	T	P	T	P	T	P	T		P	T	P	T	P	T	P	T
May 17	-	-	-	-	0	17	-	-	May 9	-	-	-	-	2	19	-	-
May 18	-	-	-	-	3	17	-	-	May 10	-	-	-	-	0	21	-	-
May 19	-	-	-	-	0	20	-	-	May 11	-	-	-	-	0	22	-	-
May 20	-	-	-	-	3	18	-	-	May 12	-	-	-	-	0	22	-	-
May 21	-	-	-	-	6	18	-	-	May 13	-	-	-	-	0	24	-	-
May 22	-	-	-	-	7	18	-	-	May 14	-	-	-	-	3	21	-	-
May 23	-	-	-	-	0	21	-	-	May 15	-	-	-	-	1	23	-	-
May 24	3	15	-	-	2	20	2	20	May 16	2	17	-	-	0	25	0	25
May 25	0	17	-	-	2	22	2	22	May 17	5	17	-	-	0	25	0	25
May 26	15	14	-	-	7	19	7	19	May 18	0	19	-	-	0	26	0	26
May 27	11	12	-	-	-	-	12	16	May 19	7	18	-	-	-	-	3	24
May 28	10	12	-	-	-	-	14	14	May 20	8	18	-	-	-	-	0	27
May 29	2	17	-	-	-	-	4	17	May 21	6	18	-	-	-	-	2	26
May 30	3	15	-	-	-	-	3	19	May 22	0	22	-	-	-	-	0	27
May 31	0	20	3	15	-	-	0	20	May 23	0	23	0	23	-	-	2	27
June 1	0	18	0	18	-	-	0	22	May 24	0	23	0	23	-	-	1	26
June 2	7	16	7	16	-	-	0	23	May 25	0	24	0	24	-	-	0	25
June 3	-	-	4	16	-	-	-	-	May 26	-	-	5	23	-	-	-	-
June 4	-	-	2	18	-	-	-	-	May 27	-	-	6	22	-	-	-	-
June 5	-	-	0	21	-	-	-	-	May 28	-	-	8	22	-	-	-	-
June 6	-	-	0	20	-	-	-	-	May 29	-	-	5	24	-	-	-	-
June 7	-	-	2	21	-	-	-	-	May 30	-	-	0	25	-	-	-	-
June 8	-	-	1	22	-	-	-	-	May 31	-	-	1	25	-	-	-	-
June 9	-	-	0	23	-	-	-	-	June 1	-	-	0	27	-	-	-	-

<sup>a</sup>Abbreviations: DA PRE-T - days after pre transplantation; POST-T (OT) - post transplantation (over-the-top); P- precipitations; T-temperature

Temperatures particularly in 2021 for the both regions were a little bit above the average (15.5 and 18.1°C). That was attributed to favorable environmental conditions associated with non-frost night time during the estimated 10 days period after PRE-T and POST-T (OT) applications. PRE-T and POST-T (OT) treatments in both years were applied at times when herbicide applications typically occur in North Macedonia tobacco production (Tab. 2) and are thus representative of producer practices and label recommendations.

### Statistical Analysis

Tests were conducted to assess the homogeneity of variance as well as the normality of distribution of the data (Ramsey and Schafer, 1997). In order to achieve approximately equal variances and better symmetry, log transformation was applied before ANOVA was performed. The control of annual weeds, the injury to tobacco, and the yield data were examined utilizing a linear mixed model (LMM) through the lmer function in the LME4 package version 1.1–23 (Bates et al. 2015), in conjunction with ANOVA at a significance level of 5%. The data regarding annual weeds (%) for the weedy control were excluded from the analysis because the values being recorded as 0% (Sarangi and Jhala 2018). The analysis of injury and tobacco yield included both the weedy control and the weed-free control. All weed data were analyzed by weed species, and site-years were pooled by weed species present at each site. During the tobacco cultivation periods of 2020 and 2021, the variables of location, herbicide treatment, year, and the interaction between PRE-T and POST-T (OT) treatment, as well as year, were treated as fixed effects, while the replications for each year were considered as random effects. For the tobacco growing seasons of 2020 and 2021, location, herbicide treatment, year, and the interaction between PRE-T and POST-T (OT) treatment, and year were treated as fixed effects, while replications within each year were regarded as random effects. The interaction of treatment by year was examined for each response variable to evaluate variability over the years. At last, the data were converted back to their original scale for the purpose of presentation. Means were differentiated through the LSD test at a 5% probability level. Statistical analysis was performed using R statistical software version 4.2.2 (R Core Team, 2022) using the glmmTMB package (function *glmmTMB*; Brooks et al. 2017).

## RESULTS AND DISCUSSION

Both sites were naturally infested with a high population of AMARE, POROL, CHEAL, POLAV and DIGSA. With the exception of DIGSA, grass weed infestations remained light throughout both years of the experiment. In the nontreated control plots, the weed density was 305 plants/m<sup>2</sup> in 2020 and 267 plants/m<sup>2</sup> in 2021 in the Prilep region, whereas in the Titov Veles region, it was 209 plants/m<sup>2</sup> in 2020 and 87 plants/m<sup>2</sup> in 2021.

### Weed control

The effectiveness of PRE-T and POST-T (OT) herbicides differed across various species of weeds, the treatments applied, the regions analyzed, and the years studied, respectively. The performance of the PRE-T and POST-T (OT) herbicides was linked to the prevailing weather and soil conditions. The variations in weather conditions over the two-year study period probably affected the effectiveness of herbicide treatments. The humid conditions of May 2020 (Tab. 2), especially with the 10 DA PRE-T application (51 mm) prior to weed emergence, led to the leaching of PRE-T herbicides from the soil surface. This occurrence was likely the primary reason for the reduced efficacy of PRE-T

herbicides when compared to their application as POST-T (OT) treatment in the Prilep region during 2020 (Tab. 3). In general, a significant amount of rainfall and heavy precipitation occurring shortly after the application of herbicides to the soil, particularly in sandy soils with low organic matter (Inoue *et al.* 2010; WSSA, 2014), such as the fluvisol sandy loam found in the Prilep region, can lead to the leaching of herbicides through the soil profile, moving them away from the area where weed seeds germinate, thereby reducing the effectiveness of the herbicides (Heatherly and Hodges, 1999; Ferrell *et al.* 2004). In 2020, Titov Veles experienced an unusual amount of precipitation (28 and 25 mm, respectively), as well. However, because of their equal occurrence during the 10 DA PRE-T and POST-T (OT) application, along with the characteristics of the vertisol soil type (which contains a higher level of clay and organic matter), leaching did not occur, leading to good efficacy for PRE-T and excellent efficacy for POST-T (OT).

The precipitation observed in 2021 within the 10 DA PRE-T applications aligned with the average for the Prilep region. Nonetheless, the precipitation recorded 10 DA POST-T (OT) application exceeded the 30-year average for this region (44 mm), resulting in considerable leaching of herbicides and a decrease in the effectiveness of POST-T (OT) herbicides. Contrary, the restricted rainfall following the PRE-T and the POST-T (OT) application (6 and 8 mm, respectively over 10 DA of both applications, may have contributed to the inadequate performance of PRE-T and POST-T (OT) herbicide treatments in the Titov Veles region in 2021 relative to 2020 (Tab. 4). Due to the ability of many soil-applied herbicides to volatilize and photodegrade on the soil surface over time, precipitation is necessary to transport these herbicides into the zone where weed seeds germinate (Wilcut *et al.* 1994; Janak and Grichar, 2016). This explains the inconsistent control of the dominant weeds observed with PRE-T and POST-T (OT) herbicide applications during the drought conditions studied in the Titov Veles region in mid-spring 2021. The variations in efficacy were not solely attributed to inconsistent weather conditions during the two years of the study, but also to the operational method of the tobacco seedling transplanting machine, as well (Fei and YaMin, 2018). In the process of transplanting seedlings, following the application of PRE-T herbicides, the machine's furrow plough extracts the non-treated soil layer, which is abundant in weed seeds, thus covering the treated layer below. Consequently, new weed emergence was detected in the tobacco rows during the PRE-T treatment, whereas this was not observed in the POST-T (OT) herbicide treatment.

***Digitaria sanguinalis*** (DIGSA). A significant treatment by treatment and treatments by year interaction resulted in two distinct years for control of DIGSA in Prilep region (Tab. 3). In 2020 efficacy of PRE-T herbicides was not satisfactory due to higher amounts of precipitation (51 mm) occurred 10 DA PRE-T application. They provided no more than 76% control of DIGSA. Significant increasing in DIGSA control herbicides provided in POST-T (OT) treatment. Except metobromuron which controlled DIGSA 91%, the rest of the

herbicides controlled this weed between 95 and 100%. In 2021 efficacy of PRE-T herbicides in control of DIGSA was ranged between 77% (metobromuron) and 90% (clomazone). Significant decreasing in control of this weed occurred in POST-T (OT) treatment due to high quantity of rainfall during 10 DA POST-T (OT) application; efficacy was ranged from 61% (metobromuron) to 74% (pendimethalin) (Tab. 3). In investigation of Byrd and York (1987), pendimethalin provides excellent (>90%) control of DIGSA in cotton. Similarly, Boydston et al. (2010) found that no DIGSA appeared in switchgrass plots treated with PRE pendimethalin.

***Chenopodium album*** (CHEAL). A significant treatment by treatment and treatments by year interaction resulted in two distinct years for control of CHEAL in Prilep region (Tab. 3). In this region, in 2020 all PRE-T applied herbicides provided inadequate control of CHEAL; efficacy was ranged between 61% (S-metolachlor) and 73% (pendimethalin). CHEAL control was significantly increased in POST-T (OT) treatment; investigated herbicides provided effective control of CHEAL (>94%). In 2021, PRE-T applied herbicides controlled CHEAL between 78% (dimethenamid) and 88% (metobromuron). Substantial decreasing in herbicides efficacy was recorded in POST-T (OT) treatment, when CHEAL was controlled no more than 73% (Tab. 3). A significant treatment by treatment in 2020 and treatments by year interaction resulted in two distinct years for control of CHEAL in Titov Veles region (Tab. 4). Herbicides provided good to excellent CHEAL control in 2020. This weed was controlled between 78% (metobromuron) and 85% (S-metolachlor) with PRE-T treatment, but significant increasing in efficacy (>97%) was recorded in POST-T (OT) herbicides application. Contrary, in 2021, due to dry soil conditions, control of CHEAL was less than 74% and 79%, with PRE-T and POST-T (OT) treatment, respectively (Tab. 4). Application of pendimethalin at 1.5 kg/ha as pre-plant and pre-emergence resulted in significantly lower population and dry weight of dominant weeds like CHEAL in chickpea (Balyan *et al.* 1987). CHEAL was controlled by 91-100% by pendimethalin PRE at 1100 g/ha in maize (Chomas and Kells, 2004). Similar, in banana pepper S-metolachlor at 534 g ai ha<sup>-1</sup> provided control of CHEAL of 99 (2 WAT) and 85 (4 WAT), while S-metolachlor at 1,070 g ai ha<sup>-1</sup> provided control of CHEAL of 96 (2 WAT) and 90 (4 WAT) (Mohseni-Moghadam and Doohan, 2015).

***Amaranthus retroflexus*** (AMARE). Two different years for AMARE control were found in both regions as a result of a significant treatments by year interaction. However, AMARE control did not significantly differ among treatments in 2021 in Titov Veles region. In 2020, in Prilep region PRE-T control of AMARE was poor and ranged of 58% (clomazone) to 74% (dimethenamid). In POST-T (OT) treatment herbicide efficacy in control of AMARE was substantially higher. Unless clomazone, which poorly controlled AMARE (79%), the rest of the herbicides nearly fully controlled this weed (>96%). The next 2021 efficacy of herbicides applied PRE-T was ranged between 67% (clomazone) and 87% (metorbomuron). Significantly reducing of AMARE control efficacy was

recorded in POST-T (OT) treatment. The herbicides provided control between 58% (clomazone), and 73% (metobromuron) (Tab. 3). In Titov Veles region, in the first experimental year (2020), PRE-T herbicides controlled AMARE between 71% (clomazone) and 85% (aclonifen). Significantly increased control of AMARE herbicides provided in POST-T (OT) treatment. Except clomzone (80%), the rest of the herbicides provided AMARE control >99%. In the second experimental year (2021), efficacy of both treatments substantially decreased; PRE-T and POST-T (OT) herbicide treatments gave only marginal control of AMARE (<73% and <76%, respectively) (Tab. 4). Preplant-incorporated pendimethalin at 1080 g ai ha<sup>-1</sup> provided excellent (95-98%) control of AMARE and reduced density 97% and dry weight 99% compared to the weedy check in white bean in Ontario (Soltani *et al.* 2013). Dimethenamid-P applied alone gave excellent control (>98%) of AMARE in dry bean (Arnold *et al.* 2012). Similar, in sugarbeet crop dimethenamid-P applied at 0.84 kg/ha and S-metolachlor at 1.4 kg/ha controlled AMARE >98% (Bollman and Sprague, 2007).

***Portulaca oleracea*** (POROL). A significant treatment by treatment and treatments by year interaction resulted in two distinct years for control of POROL in Prilep region (Tab. 3). In this region, in 2020 all PRE-T applied herbicides provided insufficient control of POROL; efficacy was ranged between 69% (S-metolachlor) and 60% (pendimethalin). POROL control was significantly improved in POST-T (OT) treatment; herbicides provided effective control of POROL (>93%). In 2021, PRE-T applied herbicides controlled POROL between 78% (clomazone) and 86% (S-metolachlor). Substantial decreasing in herbicides efficacy was recorded in POST-T (OT) treatment, when POROL was controlled no more than 72% (Tab. 3). A significant treatment by treatment in 2020 and treatments by year interaction resulted in two distinct years for control of POROL in Titov Veles region (Tab. 4). Investigated herbicides provided good (in PRE-T treatment) to excellent (in POST-T (OT) treatment) control of POROL in 2020. This weed was controlled between 77% (dimethenamid) and 87% (metobromuron), in PRE-T, and 97% (pendimethalin) and 100% (metobromuron, clomazone and aclonifen) in POST-T (OT) treatment. Opposite, in 2021 control of POROL was less than 76% and 79%, with PRE-T and POST-T (OT) treatment, respectively (Tab. 4). Preemergence control of POROL in leafy greens with S-metolachlor 0.45 and 0.67kg ha<sup>-1</sup>, and pendimethalin 0.43 and 0.86 kg ha<sup>-1</sup> resulted in >84% control (Norsworthy and Smith, 2005). An evaluation of PRE herbicides for weed control in pumpkin found that 21 days after treatment, dimethenamid 2.24 kg ha<sup>-1</sup>, and clomazon + sulfentrazone 0.37 + 0.28 kg ha<sup>-1</sup> resulted in 81-100%, and 82-98% control of POROL, respectively (Brown and Masiunas, 2002). Aclonifen gave good total weed control, with values of efficacy between 81 and 88% due to good efficacy against POROL the main species in the sorghum crop (Pannacci and Bartolini, 2018).

***Polygonum aviculare*** (POLAV). A significant treatment by year interaction resulted in two distinct years for POLAV control in Titov Veles

region. However, POLAV control did not significantly differ among treatments in 2021 in this region (Tab. 4).

**Table 3.** *Digitaria sanguinalis*, *Chenopodium album*, *Amaranthus retroflexus*, and *Portulaca oleracea* control (%) 28 days after PRE-T and POST-T (OT) herbicide applications in tobacco in 2020 and 2021 in Prilep region <sup>a-d</sup>

		Prilep region															
Treatments	Rate L/ha	DIGSA				CHEAL				AMARE				POROL			
		2020		2021		2020		2021		2020		2021		2020		2021	
		PRE-T	POST-T (OT)	PRE-T	POST-T (OT)	PRE-T	POST-T (OT)	PRE-T	POST-T (OT)	PRE-T	POST-T (OT)	PRE-T	POST-T (OT)	PRE-T	POST-T (OT)	PRE-T	POST-T (OT)
		28 DA PRE-T and POST-T (OT) A															
Weedy control	-----	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pendimethalin	3.0	76 <sup>a</sup>	98 <sup>a</sup>	87 <sup>a</sup>	74 <sup>a</sup>	73 <sup>a</sup>	96 <sup>bc</sup>	84 <sup>ab</sup>	71 <sup>ab</sup>	70 <sup>ab</sup>	98 <sup>ab</sup>	84 <sup>a</sup>	70 <sup>ab</sup>	60 <sup>c</sup>	93 <sup>c</sup>	81 <sup>ab</sup>	67 <sup>abc</sup>
Metobromuron	3.0	56 <sup>c</sup>	91 <sup>c</sup>	77 <sup>b</sup>	61 <sup>c</sup>	64 <sup>b</sup>	98 <sup>ab</sup>	88 <sup>a</sup>	73 <sup>a</sup>	63 <sup>cd</sup>	100 <sup>a</sup>	87 <sup>a</sup>	73 <sup>a</sup>	64 <sup>bc</sup>	96 <sup>b</sup>	84 <sup>a</sup>	70 <sup>a</sup>
Clomazone	1.0	74 <sup>a</sup>	100 <sup>a</sup>	90 <sup>a</sup>	72 <sup>a</sup>	70 <sup>a</sup>	96 <sup>bc</sup>	82 <sup>abc</sup>	67 <sup>bc</sup>	58 <sup>d</sup>	79 <sup>c</sup>	67 <sup>c</sup>	58 <sup>c</sup>	62 <sup>bc</sup>	97 <sup>b</sup>	78 <sup>b</sup>	63 <sup>c</sup>
Aclonifen	2.5	63 <sup>b</sup>	95 <sup>b</sup>	78 <sup>b</sup>	65 <sup>bc</sup>	67 <sup>a</sup>	100 <sup>a</sup>	80 <sup>bc</sup>	64 <sup>cd</sup>	69 <sup>ab</sup>	99 <sup>a</sup>	81 <sup>ab</sup>	69 <sup>ab</sup>	72 <sup>a</sup>	100 <sup>a</sup>	83 <sup>ab</sup>	72 <sup>a</sup>
S-metolachlor	1.5	73 <sup>a</sup>	100 <sup>a</sup>	86 <sup>a</sup>	69 <sup>ab</sup>	61 <sup>b</sup>	94 <sup>c</sup>	81 <sup>bc</sup>	61 <sup>d</sup>	66 <sup>bc</sup>	100 <sup>a</sup>	78 <sup>b</sup>	65 <sup>abc</sup>	69 <sup>a</sup>	100 <sup>a</sup>	86 <sup>a</sup>	65 <sup>bc</sup>
Dimethenamid	1.7	70 <sup>a</sup>	100 <sup>a</sup>	82 <sup>b</sup>	73 <sup>a</sup>	69 <sup>a</sup>	96 <sup>bc</sup>	78 <sup>c</sup>	65 <sup>cd</sup>	74 <sup>a</sup>	96 <sup>b</sup>	76 <sup>b</sup>	63 <sup>bc</sup>	67 <sup>ab</sup>	95 <sup>bc</sup>	80 <sup>ab</sup>	69 <sup>ab</sup>
LSD 0.05		6.63	3.56	5.81	5.57	7.20	3.33	6.76	5.56	5.87	2.72	6.78	7.78	6.42	2.79	6.69	5.61
Random effect interactions																	
PRE-T x POST-T (OT) herbicides treatment		*		*		*		*		*		*		*		*	
Random effect interactions																	
PRE-T and POST-T (OT) herbicide treatments x year			*				*				*				*		

<sup>a</sup>Abbreviation: PRE-T- (pre transplantation) and POST-T-OT (post transplantation over-the-top); DAA-days after application; DIGSA – *Digitaria sanguinalis*, POROL – *Portulaca oleracea*; AMARE - *Amaranthus retroflexus*; CHEAL - *Chenopodium album*; NS-not significant; \* Significant at the 5% level according to a Fisher's protected LSD test at P<0.05.

<sup>b</sup>PRE-T treatments were applied two days before tobacco transplantation. POST-T (OT) treatments were applied five days after tobacco transplantation

<sup>c</sup>Weed control efficacy was estimated 28 DAA

<sup>d</sup>Means followed by the same letter within a column are not significantly different according to Fisher's Protected LSD at P<0.05

**Table 4.** *Amaranthus retroflexus*, *Portulaca olearacea*, *Chenopodium album* and *Polygonum aviculare* control (%) 28 days after PRE-T and POST-T (OT) herbicide applications in tobacco in 2020 and 2021 in Titov Veles region <sup>a-</sup>

		Titov Veles region															
Treatments	Rate L/ha	AMARE				POROL				CHEAL				POLAV			
		2020		2021		2020		2021		2020		2021		2020		2021	
		PRE-T	POST-T (OT)	PRE-T	POST-T (OT)	PRE-T	POST-T (OT)	PRE-T	POST-T (OT)	PRE-T	POST-T (OT)	PRE-T	POST-T (OT)	PRE-T	POST-T (OT)	PRE-T	POST-T (OT)
28 DA PRE-T and POST-T (OT) A																	
Weedy control	-----	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pendimethalin	3.0	83 <sup>a</sup>	99 <sup>a</sup>	69 <sup>ab</sup>	72 <sup>ab</sup>	83 <sup>ab</sup>	97 <sup>b</sup>	66 <sup>c</sup>	69 <sup>c</sup>	79 <sup>a</sup>	97 <sup>b</sup>	69 <sup>ab</sup>	75 <sup>abc</sup>	84 <sup>ab</sup>	98 <sup>a</sup>	74 <sup>abc</sup>	76 <sup>ab</sup>
Metobromuron	3.0	79 <sup>a</sup>	100 <sup>a</sup>	71 <sup>ab</sup>	75 <sup>a</sup>	87 <sup>a</sup>	100 <sup>a</sup>	69 <sup>bc</sup>	75 <sup>ab</sup>	78 <sup>a</sup>	100 <sup>a</sup>	72 <sup>ab</sup>	77 <sup>ab</sup>	81 <sup>ab</sup>	100 <sup>a</sup>	67 <sup>c</sup>	72 <sup>b</sup>
Clomazone	1.0	71 <sup>b</sup>	80 <sup>b</sup>	60 <sup>c</sup>	67 <sup>b</sup>	86 <sup>a</sup>	100 <sup>a</sup>	71 <sup>abc</sup>	74 <sup>abc</sup>	83 <sup>a</sup>	98 <sup>b</sup>	74 <sup>a</sup>	79 <sup>a</sup>	86 <sup>a</sup>	100 <sup>a</sup>	77 <sup>a</sup>	79 <sup>a</sup>
Aclonfen	2.5	85 <sup>a</sup>	100 <sup>a</sup>	73 <sup>a</sup>	76 <sup>a</sup>	79 <sup>b</sup>	100 <sup>a</sup>	74 <sup>ab</sup>	77 <sup>ab</sup>	82 <sup>a</sup>	100 <sup>a</sup>	67 <sup>b</sup>	70 <sup>c</sup>	80 <sup>ab</sup>	99 <sup>a</sup>	72 <sup>abc</sup>	75 <sup>ab</sup>
S-metolachlor	1.5	78 <sup>ab</sup>	100 <sup>a</sup>	66 <sup>bc</sup>	70 <sup>ab</sup>	86 <sup>a</sup>	98 <sup>b</sup>	70 <sup>abc</sup>	72 <sup>bc</sup>	85 <sup>a</sup>	100 <sup>a</sup>	70 <sup>ab</sup>	73 <sup>bc</sup>	77 <sup>b</sup>	95 <sup>b</sup>	70 <sup>abc</sup>	78 <sup>a</sup>
Dimethenamid	1.7	81 <sup>a</sup>	99 <sup>a</sup>	67 <sup>ab</sup>	74 <sup>a</sup>	77 <sup>b</sup>	97 <sup>b</sup>	76 <sup>a</sup>	79 <sup>a</sup>	80 <sup>a</sup>	100 <sup>a</sup>	67 <sup>b</sup>	71 <sup>c</sup>	83 <sup>ab</sup>	100 <sup>a</sup>	69 <sup>bc</sup>	74 <sup>ab</sup>
LSD 0.05		7.12	1.65	6.94	6.40	6.96	1.70	6.58	5.95	7.95	1.37	6.41	5.32	7.44	2.12	7.42	5.27
Random effect interactions PRE-T x POST-T (OT) herbicides treatment		*		NS		*		NS		*		NS		*		NS	
Random effect interactions PRE-T and POST-T (OT) herbicide treatments x year			*				*				*				*		

<sup>a</sup>Abbreviation: PRE-T- (pre transplantation) and POST-T-OT (post transplantation over-the-top); DAA-days after application; AMARE - *Amaranthus retroflexus*; POROL - *Portulaca olearacea*; CHEAL - *Chenopodium album*, POLAV – *Polygonum aviculare*; NS-not significant; \*Significant at the 5% level according to a Fisher's protected LSD test at P<0.05. <sup>b</sup> PRE-T treatments were applied two days before tobacco transplantation. <sup>c</sup> POST-T (OT) treatments were applied five days after tobacco transplantation. Weed control efficacy was estimated 28 DAA d Means followed by the same letter within a column are not significantly different according to Fisher's Protected LSD at P<0.05

In the first experimental year (2020), PRE-T herbicides controlled POLAV between 77% (S-metolachlor) and 86% (clomazone). Significantly increased control of POLAV herbicides provided in POST-T (OT) treatment. They controlled this weed more than 95%. In the second experimental year (2021), efficacy of both treatments substantially decreased; PRE-T and POST-T (OT) herbicide treatments gave only marginal control of POLAV (<77% and <79%, respectively) (Tab. 4). In black locust (*Robinia pseudoacacia* L.) nurseries in 2010, S-metolachlor and pendimethalin controlled POLAV ≥90% 28 DAT and 56 DAT, respectively. Lower herbicide efficacy was noticed in 2011 (between 75

and 83%) most likely due to high precipitation occurred immediately after herbicide application (Pacanoski *et al.* 2016).

### **Tobacco injury**

In North Macedonia's tobacco production, PRE-T and POST-T (OT) herbicides application coincided with the period when herbicide applications are usually performed, thereby reflecting practices of producers and label guidelines. However, in 2020 in Prilep region heavy precipitation occurred 10 DA PRE-T, caused leaching of PRE-T herbicides through the soil profile. It is highly possible that tobacco injury was due to higher amounts of rain directly following PRE-T herbicides treatment. The range was between 10% and 30% following the PRE-T treatment, recorded 7 days post herbicide application (DAHA). The extent of injuries from pendimethalin and S-metolachlor was greater, with rates of 28% and 30%, respectively. In both herbicide treatments, the symptoms observed included stunted, yellowing plants, and at times, mottled and distorted lower leaves were also present. The impact of other PRE-T herbicides on tobacco injuries was reduced by 7 and 21 DAHA. Still, the tobacco damage caused by pendimethalin and S-metolachlor was evident at 21 DAHA. During the POST-T (OT) treatment, minor tobacco injuries were noted; however, their significance was minimal. In 2021, precipitation above the 30-year average for this region was recorded 10 DA POST-T (OT) treatment, resulting in greater tobacco injury. It ranged from 12 to 27% across POST-T (OT) treatment 7 DAHA. Injuries caused by pendimethalin and S-metolachlor were again more severe, with rates of 26% and 27%, respectively, and were still apparent 21 DAHA (19 and 22%, respectively) (Tab. 5). Nevertheless, tobacco demonstrated a relatively rapid recovery from these injuries, as the symptoms associated with the injuries were no longer present by the end of the second harvest. In the region of Titov Veles, only minimal tobacco injuries were noted over the two experimental years, specifically concerning POST-T (OT) treatment. At 21 DAHA, all herbicides resulted in 12% or less injury (Tab. 6). POST-T trifloxysulfuron, halosulfuron and sulfentrazone caused substantial dark tobacco injury of 16 to 39% at 1 wk after treatment (WAT), but tobacco injury from POST-T applications had dissipated to 6 to 12% by 4 WAT (Bailey, 2007). Similar to ours are investigation of Yousafzai *et al.* (2006) who concluded that S-metolachlor showed a phytotoxic effect on tobacco in form of retarded plant growth. S-metolachlor at 1.07 and 2.14 kg a.i. ha<sup>-1</sup> applied PTI injured tobacco plants 6 WAT, but at the end of the season tobacco growth recovered only from smaller S-metolachlor rate (Clapp *et al.* 2020). S-metolachlor applied at 1.07 kg a.i. ha<sup>-1</sup> POST-OT exhibited 12% tobacco plants injury 7 DAHA. However, injury was with transient character (less than 2% 42 DAHA) (Clapp *et al.* 2022). In study of Fisher and Smith (2000), the highest injuries to tobacco have been observed with the use of pendimethalin. Greater rainfall through 3 WAT occurred in 2000 than in 2001 or 2002, likely contributing to the higher Maryland-type tobacco injury in 2000 caused by sulfentrazone in combination with clomazone or pendimethalin

(Ritter *et al.* 2005). Similar, pendimethalin and clomazone caused relatively minor stunting of tobacco each year (ranging from 10, 11 and 2% injury for 2005, 2006 and 2007, respectively). The heavier rainfall in 2005 and 2006 likely contributed to greater herbicide uptake by tobacco which resulted in greater crop injury (Bailey *et al.* 2014). Walker *et al.* (1998) reported sulfentrazone applied PPI and sulfentrazone plus clomazone PRE-T caused up to 26% discoloration of tobacco 15 and 30 d after treatment (DAT). Breeden *et al.* (1999) examined combinations of sulfentrazone with clomazone or pendimethalin either applied to the surface or incorporated, and reported tobacco injury at one location was 20% or less 15 DAT with a slight increase in injury with incorporation. At 64 DAT, all treatments exhibited 5% or less injury. No injury from S-metolachlor applied at 1.07 kg a.i. ha<sup>-1</sup> POST-T (OT) before the first harvest was reported by Whaley (2017).

### **Tobacco yield**

Overall effectiveness of weed control and the extent of crop injury were generally reflected in the tobacco yields for each treatment across both regions. (Tab. 5 and 6). A comparison between weedy and weed-free control showed that the presence of weeds decreased tobacco yield by 57-65% in the Prilep region, and by 60-62% in the Titov Veles region across both years, respectively. (Tab. 5 and 6). Travlos *et al.* (2014) concluded no weed management at all resulted in lower yields up to 43 %. Dhanapal *et al.* (1998) reported that the use of herbicides increased the tobacco yield by 80 to 100% when compared to control treatment. Treatments with metolachlor, metobromuron, napropamide and trifluralin increased tobacco yields (averaged over years) for 36.1, 29.5 and 37.9%, respectively compared with untreated control (Dimeska *et al.* 2003). A considerable treatment by treatment interaction resulted in two different years for tobacco yield in the Prilep region. Nevertheless, the yield of tobacco did not exhibit any significant differences in relation to the interaction between treatment and year. Across both years, the lowest yield of tobacco, without consideration of treatment (PRE-T or POST-T (OT)), was noted in the untreated control plots (1250 and 1440 kg/ha, respectively). As a result of leaching and inadequate weed control, the overall yield in plots treated with PRE-T herbicide varied between 2360 and 2510 kg/ha, compared to a weed-free control which yielded 3560 kg/ha. Within the plots that were treated with herbicides, the highest total yield was recorded for tobacco treated with aclonifen, while the lowest total yield was observed in the tobacco treated with S-metolachlor. In the POST-T (OT) treatment, the yields aligned closely with those of the weed-free control, ranging from 1250 to 3560 kg/ha. Among the herbicide treatments, metobromuron resulted in the lowest yield of 3420 kg/ha, whereas S-metolachlor and dimethenamid provided the highest yield of 3510 kg/ha. The differences observed between metobromuron, from one side, and S-metolachlor and dimethenamid, from another, are likely related to differences in the extent of tobacco injuries and the efficacy of weed control. In investigation of Yousafzai *et al.* (2006) between herbicides maximum yield of 2465 kg/ha was recorded in pendimethalin (EC)

treatment and minimum yield of 1788 kg/ha in S-metolachlor treated plots. In the experimental 2021, the total yield achieved through PRE-T herbicides treatment was between 3010 kg/ha (aclonifen) and 3140 kg/ha (S-metolachlor), compared to 3350 kg/ha yield recorded in the weed-free control. The considerable differences between the weed-free control and the PRE-T herbicides treatment were likely attributed to the emergence of new weeds. This phenomenon is likely a result of the transplanting machine's operational method, particularly the furrow plough, which dislodges the untreated soil layer rich in weed seeds on the surface, subsequently covering the treated soil. The leaching of herbicides, which led to a decrease in weed control and injuries to tobacco in POST-T (OT) treatment, resulted in a yield that was considerably lower when compared to the weed-free control and the treatment with PRE-T herbicides (Tab.5).

**Table 5.** Tobacco plant injury as influenced by PRE-T and POST-T (OT) applied herbicides, and grain yield as influenced by PRE-T and POST-T (OT) applied herbicides in tobacco in Prilep region in 2020 and 2021 <sup>a-d</sup>

Treatments	Prilep region											
	Tobacco injury								Tobacco yield (kg/ha)			
	2020				2021				2020		2021	
	PRE-T		POST-T (OT)		PRE-T		POST-T (OT)		PRE-T	POST-T (OT)	PRE-T	POST-T (OT)
	7 DAHA	21 DAHA	7 DAHA	21 DAHA	7 DAHA	21 DAHA	7 DAHA	21 DAHA				
Weedy control	0 <sup>d</sup>	0 <sup>d</sup>	0 <sup>d</sup>	0 <sup>b</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>c</sup>	1250 <sup>d</sup>	1250 <sup>c</sup>	1440 <sup>d</sup>	1440 <sup>a</sup>
Weed-free control	0 <sup>d</sup>	0 <sup>d</sup>	0 <sup>d</sup>	0 <sup>b</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>d</sup>	0 <sup>c</sup>	3560 <sup>a</sup>	3560 <sup>a</sup>	3350 <sup>a</sup>	3350 <sup>a</sup>
Pendimethalin	28 <sup>a</sup>	21 <sup>a</sup>	7 <sup>ab</sup>	2 <sup>b</sup>	2 <sup>b</sup>	0 <sup>a</sup>	26 <sup>a</sup>	19 <sup>a</sup>	2380 <sup>bc</sup>	3490 <sup>ab</sup>	3100 <sup>bc</sup>	2440 <sup>cd</sup>
Metobromuron	20 <sup>b</sup>	14 <sup>b</sup>	4 <sup>bc</sup>	0 <sup>b</sup>	1 <sup>ab</sup>	0 <sup>a</sup>	18 <sup>b</sup>	9 <sup>b</sup>	2440 <sup>bc</sup>	3420 <sup>b</sup>	3040 <sup>bc</sup>	2520 <sup>bc</sup>
Clomazone	12 <sup>c</sup>	10 <sup>bc</sup>	4 <sup>bc</sup>	1 <sup>b</sup>	0 <sup>a</sup>	0 <sup>a</sup>	14 <sup>bc</sup>	7 <sup>b</sup>	2470 <sup>bc</sup>	3480 <sup>ab</sup>	3060 <sup>bc</sup>	2560 <sup>b</sup>
Aclonifen	10 <sup>c</sup>	7 <sup>c</sup>	2 <sup>c</sup>	0 <sup>b</sup>	0 <sup>a</sup>	0 <sup>a</sup>	12 <sup>c</sup>	7 <sup>b</sup>	2510 <sup>b</sup>	3450 <sup>ab</sup>	3010 <sup>c</sup>	2480 <sup>cd</sup>
S-metolachlor	30 <sup>a</sup>	25 <sup>a</sup>	10 <sup>a</sup>	6 <sup>a</sup>	2 <sup>b</sup>	0 <sup>a</sup>	27 <sup>a</sup>	22 <sup>a</sup>	2360 <sup>c</sup>	3510 <sup>ab</sup>	3140 <sup>b</sup>	2390 <sup>d</sup>
Dimethenamid	20 <sup>b</sup>	13 <sup>b</sup>	5 <sup>bc</sup>	1 <sup>b</sup>	0 <sup>a</sup>	0 <sup>a</sup>	18 <sup>b</sup>	11 <sup>b</sup>	2420 <sup>bc</sup>	3510 <sup>ab</sup>	3120 <sup>bc</sup>	2580 <sup>b</sup>
LSD 0.05	6.27	5.69	3.16	1.73	1.08	0.00	5.88	4.25	139.29	124.82	120.78	98.25
Random effect interactions												
PRE-T x POST-T (OT) herbicides treatment	*				*				*		*	
Random effect interactions PRE-T and POST-T (OT) herbicides treatment x year	NS								NS			

<sup>a</sup>Abbreviation: PRE-T- (pre transplantation) and POST-T-OT (post transplantation over-the-top); DAHA-days after herbicide application; NS-not significant;

\*Significant at the 5% level according to a Fisher's protected LSD test at P<0.05.

<sup>b</sup>PRE-T treatments were applied two days before tobacco transplantation. POST-T (OT) treatments were applied five days after tobacco transplantation

Tobacco injury was estimated 7 and 21 days after herbicide application (DAHA)

<sup>d</sup> Means followed by the same letter within a column are not significantly different according to Fisher's Protected LSD at P<0.05

**Table 6.** Tobacco plant injury as influenced by PRE-T and POST-T (OT) applied herbicides, and grain yield as influenced by PRE-T and POST-T (OT) applied herbicides in tobacco in Titov Veles region in 2020 and 2021 <sup>a-d</sup>

Treatments	Titov Veles region											
	Tobacco injury								Tobacco yield (kg/ha)			
	2020				2021				2020		2021	
	PRE-T		POST-T (OT)		PRE-T		POST-T (OT)		PRE-T	POST-T (OT)	PRE-T	POST-T (OT)
7 DAHA	21 DAHA	7 DAHA	21 DAHA	7 DAHA	21 DAHA	7 DAHA	21 DAHA					
Weedy control	0 <sup>c</sup>	0 <sup>a</sup>	0 <sup>d</sup>	0 <sup>b</sup>	0 <sup>c</sup>	0 <sup>a</sup>	0 <sup>d</sup>	0 <sup>c</sup>	1300 <sup>c</sup>	1300 <sup>c</sup>	1220 <sup>a</sup>	1220 <sup>c</sup>
Weed-free control	0 <sup>c</sup>	0 <sup>a</sup>	0 <sup>d</sup>	0 <sup>b</sup>	3 <sup>a</sup>	0 <sup>a</sup>	0 <sup>d</sup>	0 <sup>c</sup>	3420 <sup>a</sup>	3420 <sup>a</sup>	3090 <sup>a</sup>	3090 <sup>a</sup>
Pendimethalin	2 <sup>a</sup>	0 <sup>a</sup>	10 <sup>a</sup>	6 <sup>a</sup>	1 <sup>bc</sup>	0 <sup>a</sup>	13 <sup>ab</sup>	8 <sup>a</sup>	3220 <sup>b</sup>	3290 <sup>b</sup>	2580 <sup>b</sup>	2600 <sup>b</sup>
Metobromuron	1 <sup>b</sup>	0 <sup>a</sup>	7 <sup>b</sup>	2 <sup>b</sup>	1 <sup>bc</sup>	0 <sup>a</sup>	9 <sup>bc</sup>	5 <sup>ab</sup>	3290 <sup>b</sup>	3370 <sup>ab</sup>	2690 <sup>b</sup>	2730 <sup>b</sup>
Clomazone	1 <sup>b</sup>	0 <sup>a</sup>	5 <sup>bc</sup>	2 <sup>b</sup>	0 <sup>c</sup>	0 <sup>a</sup>	9 <sup>bc</sup>	4 <sup>b</sup>	3180 <sup>b</sup>	3260 <sup>b</sup>	2560 <sup>b</sup>	2580 <sup>b</sup>
Aclonifen	0 <sup>c</sup>	0 <sup>a</sup>	4 <sup>c</sup>	0 <sup>d</sup>	1 <sup>bc</sup>	0 <sup>a</sup>	8 <sup>c</sup>	4 <sup>b</sup>	3250 <sup>b</sup>	3340 <sup>ab</sup>	2620 <sup>b</sup>	2720 <sup>b</sup>
S-metolachlor	2 <sup>a</sup>	0 <sup>a</sup>	12 <sup>a</sup>	7 <sup>a</sup>	2 <sup>ab</sup>	0 <sup>a</sup>	15 <sup>a</sup>	8 <sup>a</sup>	3250 <sup>b</sup>	3320 <sup>ab</sup>	2690 <sup>b</sup>	2660 <sup>b</sup>
Dimethenamid	0 <sup>c</sup>	0 <sup>a</sup>	6 <sup>bc</sup>	2 <sup>b</sup>	1 <sup>b</sup>	0 <sup>a</sup>	9 <sup>bc</sup>	5 <sup>ab</sup>	3220 <sup>b</sup>	3330 <sup>ab</sup>	2660 <sup>b</sup>	2690 <sup>b</sup>
LSD 0.05	0.96	0.00	2.91	2.54	1.03	0.00	4.47	3.71	121.94	117.89	143.90	154.82
Random effect interactions												
PRE-T x POST-T (OT) herbicides treatment	*				*				NS		NS	
Random effect interactions PRE-T and POST-T (OT) herbicides treatment x year	NS								*			

<sup>a</sup>Abbreviation: PRE-T- (pre transplantation) and POST-T-OT (post transplantation over-the-top); DAHA-days after herbicide application; NS-not significant;

<sup>\*</sup>Significant at the 5% level according to a Fisher's protected LSD test at  $P < 0.05$ .

<sup>b</sup>PRE-T treatments were applied two days before tobacco transplantation. POST-T (OT) treatments were applied five days after tobacco transplantation

<sup>c</sup>Tobacco injury was estimated 7 and 21 days after herbicide application (DAHA)

<sup>d</sup>Means followed by the same letter within a column are not significantly different according to Fisher's Protected LSD at  $P < 0.0$

A non-significant interaction between treatments led to two comparable years in the Titov Veles region with the application of PRE-T and POST-T (OT) herbicides. On the other hand, tobacco yields show significant variation when considering the interaction of treatments across different years. In 2020, neither the PRE-T nor the POST-T (OT) herbicide applications yielded more than the weed-free control, and the differences observed between the PRE-T and POST-T (OT) treatments, from one and the weed-free control, from the other side were not significantly greater. In 2021, due to inadequate weed management, the tobacco yields after the application of all PRE-T and POST-T (OT) herbicides were

considerably lower, averaging between -460 and -430 kg/ha, respectively, compared to the weed-free control (Tab. 6).

### CONCLUSIONS

The rainfall had a major effect on PRE-T and POST-T (OT) herbicide efficacy; higher precipitation caused herbicide leaching, particularly on fluvisol sandy loam which decreased herbicide efficacy in weed control in Prilep in 2020 and 2021, respectively. However, both PRE-T and POST-T treatments demonstrated good to excellent weed control in Titov Veles, where vertisol soil characteristics (higher content of clay and OM) were favorable and precipitation more consistent. Additionally, tobacco injury was caused by heavy rainfall, particularly after the application of metolachlor and pendimethalin. Overall, crop injury and the effectiveness of herbicide treatments were directly correlated, with the weather and soil condition in the area being a significant factor. To reduce the chance of leaching and increase herbicide efficacy, PRE-T and POST-T (OT) herbicides might be best applied during periods in which high quantity of rain is not expected. To reduce crop damage, especially during times of high precipitation, should be avoid any herbicide treatments to decrease or nullify any possibility for herbicides failure and crop injury. Since the tobacco seedling transplanting machine's performance also affected efficacy of herbicides, improving the machine's performance could lead to more reliable outcomes for crop safety and weed control. The conclusion should present a clear and concise review of experiments and results obtained, with possible reference to the enclosures.

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