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Morpho-physiological responses of maize hybrids as a function prohexadione calcium doses applied in the vegetative phase

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Abstract

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Plant growth regulator prohexadione use in maize cultivation is recent, but conflicting results have been obtained on development of its vegetative structures. The aim of this study was to assess the effects of prohexadione applied in the vegetative phase, on the response of the morphophysiological parameters of maize. The pot experiments were conducted in greenhouse, in the 2017/2018 and 2018/2019 seasons. The randomized block design was arranged in a 2x7 factorial scheme with six repetitions. The main factor was growing seasons 2017/2018 and 2018/2019, and the sub factor were the growth regulator prohexadione doses: 0; 50; 100; 150; 200; 250 and 300 g ai (active ingredient) ha^{-1}, applied on plants in V7 stage. The hybrids $AS1757_{VTPRO3}$ and $AG9025_{VTPRO3}$ were tested, and each hybrid consisted of an independent experiment.

The application of prohexadione was an effective strategy to restricte the longitudinal growth of the plants of both hybrids. In hybrid $AS1757_{VTPRO3}$, plant height was restricted up to 46% and 47%, in the 2017/2018 and 2018/2019 growing season, respectively. The reduced biological yield and kernel row number per ear in the hybrid $AS1757_{VTPRO3}$ and increased the NDVI and SPAD chlorophyll index. In hybrid $AG9025_{VTPRO3}$ there was a restriction on plant height growth of 53% and 57% in the 2017/2018 and 2018/2019 growing season, respectively. But the prohexadione allowed the linear increase of the leaf area in the hybrid $AG9025_{VTPRO3}$. The prohexadione application leads to a decreased plants and ear insertion heights, tassel and peduncle length in a manner dependent of growing season, dose and hybrid.

Keywords: gibberellins inhibitors; plant growth regulator; *Zea mays* L.

Introduction

The first commercial use of exogenous chemical substances to control growth in cereal crops occurred in the mid 1960s, when the compound 2-chloroethyl trimethylammonium chloride (CCC) was recommended for wheat cultivation, and with the advent of new synthetic chemicals in the ensuing decades, these products were widely applied (Trevizan et al., 2015).

Plant growth regulators (PGRs) are synthetic chemical subtances that change the hormonal balance of plants. They bind to plant receptors, triggering a series of primarily cellular changes, which may affect the onset of or changes in organ or tissue development. Thus, they may regulate undesirable plant growth via chemical signallers, without reducing yield (Radamacher, 2015). The group of gibberellins PGRs widely studied in monocots and dicots are commonly used in cereals to reduce stem thickness, there by lowering yield losses caused by plant lodging and altering leaf architecture byproducing shorter and wider horizontal leaves. This enables better use of environmental resources and inputs and increases agronomic yield (Souza et al., 2013).

One of these plant growth regulators is prohexadione calcium (from now on, just prohexadione), a calcium structure (3-oxide-5-oxo-4-propionylcyclohexene-3-enocarboxylate), which inhibits the biosynthesis of active gibberellic acid (Evans et al.,1999) and is effective in controlling plant growth in crops such as wheat (*Triticum aestivum* L.) (Stefen et al., 2014) and rice (*Oryza sativa* L.) (Na et al., 2011) and, increasing grapevine yield (Villar et al., 2011). In Brazil, it is applied to cotton, oats, potato, begonia, rye, barley, chrysanthemum, kalanchoe, apple, poinsiettia, wheat and triticale, with a dose of 165 g ai ha^{-1} recommended for species of the family *Poaceae* (Brasil, 2019).

However, the use of this active ingredient in maize (*Zea mays* L.) cultivation is recent and conflicting results have been obtained, particularly in the behavior of vegetative structures. Applying prohexadione to maize has been assessed experimentally. Spitzer et al. (2015) studied the effects of different doses and application times of Medax Top® (mepiquat chloride + prohexadione calcium) and observed a decline in plant height, ear insertion height and ear length, albiet with no statistical significance. However, the authors worked with a single dose of 63 g ai ha^{-1} of prohexadione in phenological stage V8-V9, 63 g ai ha^{-1} near stage VT and double doses of 63 g in V8-V9 + 63 g ai ha^{-1} near stage VT on the Richie scale (Richie et al., 1993). Similar results were obtained by Pinheiro et al. (2018) at doses of 100 and 200 g ai. ha^{-1} applied in stage V12.

The hypothesis of this study is based on the fact that applying increasing prohexadione doses, at the beginning of the accelerated elongation period of the plant growth, which occurs in stages V6 to V8 (Mendes Fagherazzi et al., 2018), decreases vegetative growth in maize crops,making the plants compact, increasing stem diameter and to changing the leaf architecture of plants, which raises chlorophyll optic sensor vegetative index values and facilitates crop management. Thus, the aim of the present study was to assess the effects of increasing prohexadione doses applied in vegetative stage V7 on the response of morphological and physiological parameters in greenhouse-grown maize hybrids with contrasting cycles.

Materials and Methods

Experiments were conducted in November 2017 and December 2018 in a greenhouse at the Center for Agroveterinary Sciences of Santa Catarina State University (27°47′34″ S 50°18′05″ W and mean altitude of 930 meters). Three seeds were planted in each pot and thinned in stage V3 leaving one plant per pot. Plastic pots (diameter =22 cm and height = cm) were filled with 5 kg of dry soil, classified as humiccambisol (Embrapa, 2013). The results of chemical and physical analysis conducted for the two years are described in Table 1.

The soil was corrected following the Chemical and Soil Fertility Commission technical recommendations (Comissão de Química e Fertilidade do Solo-RS/SC, 2016) for a population of 65000 plants ha^{-1}, aiming potential yield of 9000 kg ha^{-1}. Plants were irrigated daily during the experimental period. Pesticides were not used since the growing conditions protect the crop against pests and diseases.

Temperature and relative humidity in the greenhouse were measured using a Tenmars TM-305U data logger. The sensor was programmed to collect data every 10 minutes throughout the greenhouse experiment. Relative humidity (%) and mean temperature (°C) are presented for 2017/2018(Figure1a) and 2018/2019 growing season (Figure 1b).

Two different maize hybrids were used: the early-maturing simple hybrid AS175$_{VTPRO3}$ developed by the Agroeste Company and characterized by plants with plant height of 232 cm and ear insertion height of 116 cm, and super-early simple hybrid AG9025$_{VTPRO3}$, developed by the Agroceres company, characterized by plants with plant height of 235 cm and ear insertion of 124 cm. In order to obtain accurate management guidelines and specific pesticide recommendations, each hybrid (AS1757$_{VTPRO3}$and AG9025$_{VTPRO3}$) consisted of one experiment and was analyzed separately.

Table 1. Chemical and physical analysis of the soil. Lages, Santa Catarina state, Brazil

Growing season	Clay	V	O.M.	pH (H_2O)	P Mehlich I	K	Ca	Mg	H^++Al^{+3}	CEC (pH7)
	---------%--------				---mg dm^{-3}---		---------cmol$_c$ dm^{-3}---------			
2017/2018	54	60	5.4	5.4	24.5	114	6.8	4.4	7.7	19.3
2018/2019	41	77	3.8	5.5	27.6	107	7.8	3.1	3.4	14.8

V = base saturation; O.M. = organic matter; P Mehlich-I = South Brazilian official soil P extraction; CEC cation exchange concentration

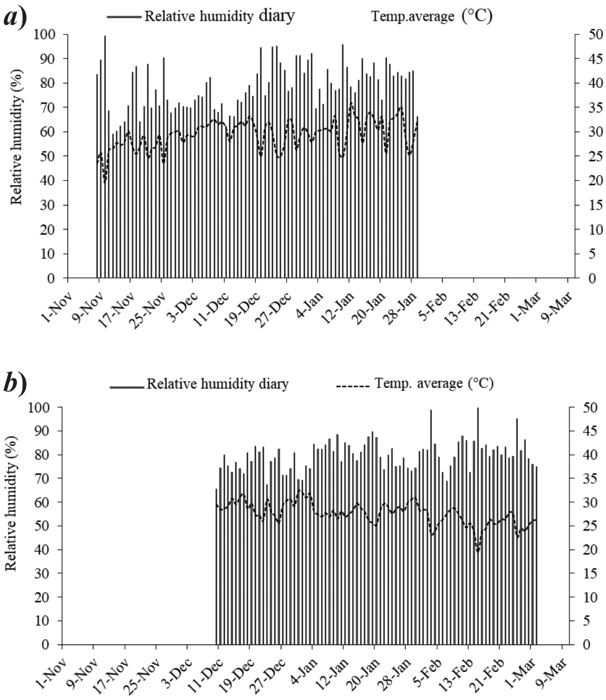

Fig. 1. Average daily relative humidity (%) and temperature in the greenhouse during the maize growing seasons of *a*) 2017/2018 (seeding at November 8th; harvest at January 29th) and *b*) 2018/2019 (seeding at December 10th; harvest at march 4th)

The randomized block design was arranged in a 2x7 factorial scheme with six repetitions. The first factor is represented by growing seasons 2017/2018 and 2018/2019, and the second consisted of prohexadione calcium doses.

Prohexadione calcium (Viviful® WG) was applied once, when the plants were in phenological stage with seven fully expanded leaves (V7) at the following doses: (control – with no plant growth regulator applied); 50; 100; 150; 200; 250 and 300 g ai ha^{-1}. The upper leaves of plants were sprayed at a constant pressure of 30 lb pol^{-2} using a CO_2-pressurized sprayer with XR 110-015 flat spray tip nozzles calibrated for a spray volume of 200 L ha^{-1}.

Plant height in the vegetative stage (PHVS) was determined 0, 7, 14 and 21 days after application (DAA) of prohexadione. PHVS was measured from the distance between ground level and the tip of the leaf blade of the last fully expanded leaf, using a tape measure graduated in centimeters.

In addition, the normalized difference vegetation index (NDVI) was determined in phenological stage VT (bolting). NDVI readings were conducted in the last fully expanded leaf, using a PlantPen NDVI-300 portable sensor, which uses wavelengths between 660 nm and 740 nm. The NDVI algorithm subtracts the red reflectance values from the near-infrared and divides the result by the sum of near-infrared and red bands. Relative chlorophyll content was determined in stage R1 (bolting and flowering), with a SPAD (Soil Plant Analysis Development) 502 plus Minolta® Konica chlorophyll meter, via readings of the middle third of the leaf index (below the ear).

The following variables were assessed in the same stage (R1): plant height (PH), distance from ground level and the tip of the tassel; earinsertion height (EIH), distance from ground level to the base of the main ear, both measured with a tape measure graduated in centimeters; tassel length (TL) and peduncle length (PDL), measured with a metric ruler; stem diameter (SD), determined on the second internode above the ground, measured with a digital pachymeter and expressed in millimeters; and the number of leaves (NL), based on the number of leaves from the base to the last fully expanded leaf at the apex of the each plant, adding photosynthetically active senescent leaves.

Leaf area was determined on harvest day when the plants were in the grain filling stage (R2) by measuring the length (L) from the base to the tip of the leaf and the largest width (W) of all the photosynthetically active leaves, according to the methodology used by Tollenaar (1992). Leaves whose ar-

Table 2. Analysis of variance (MS – Mean Square) and significance for plant height in the vegetative stage (PHVS) of two maize hybrids, analyzed at 0, 7, 14 and 21 days after prohexadione application (DAA)

SOV	DF	MS – PHVS (cm)	
		$AS1757_{VTPRO3}$	$AG9025_{VTPRO3}$
Block	5	86.95^{ns}	360.94^{ns}
Year (A)	1	81 205.21**	124 432.01**
Error A	5	446.00	535.39
Doses (D)	6	5042.16**	1202.17**
A x D	6	3580.67**	245.70^{ns}
Error B	60	181.44	182.29
DAA	3	10 052.36**	11 730.75**
A x DAA	3	379.87**	1012.40**
D x DAP	18	460.65**	284.67**
A x D x DAA	18	227.78**	96.86**
Error C	210	16.35	16.18
Overall Mean	–	125.71	130.78
CV[1] (%)	–	16.80	17.69
CV[2] (%)	–	10.71	10.32
CV[3] (%)	–	3.22	3.08

** e * = statistically different according to the F-test at ($p < 0.01$) and ($p < 0.05$), respectively; ns: not significant; SOV = sources of variation; DF = degrees of freedom; CV% = coefficient of variation eas were at least 50% green were considered photosynthetically active, in line with the criterium proposed by Borrás et al. (2003). Leaf area (LA), expressed in cm², was estimated by applying the following formula: LA = L x W x 0.75, the latter being the correction coefficient, and the sum of the areas of all green leaves determined the leaf area per plant. The following variables were assessed: kernel row number (KRN) per ear, by direct count, and biological yield (BY) per plant, obtained from shoot (leaves and stem) weight measured on a semi-analytical balance, accurate to 0.01 g, after drying in a forced air circulation oven at 65°C until constant weight.

The data were submitted to analysis of variance using the F-test at a 5% significance level. When significant, the means of the growing seasons were compared using Tukey's test (p<0.05) and the plant growth regulator (PGR) doses were submitted to polynomial regression analysis at 5% (p<0.05). For repeated measures analysis over time of plant height in the vegetative stage, the mathematical model of split-split plots in randomized blocks was considered, adopting the following procedure: growing seasons were allocated to the main plots, prohexadione doses to the sub plots and repeated measures over time to the sub-sub plots.

Results

With respect to the performance of experiments conducted with hybrids AS1757$_{VTPRO3}$ and AG9025$_{VTPRO3}$, significant effects were observed for triple interaction (growing season, doses and days after application of prohexadione) in terms of plant height in the vegetative stage (PHVS) (Ta-

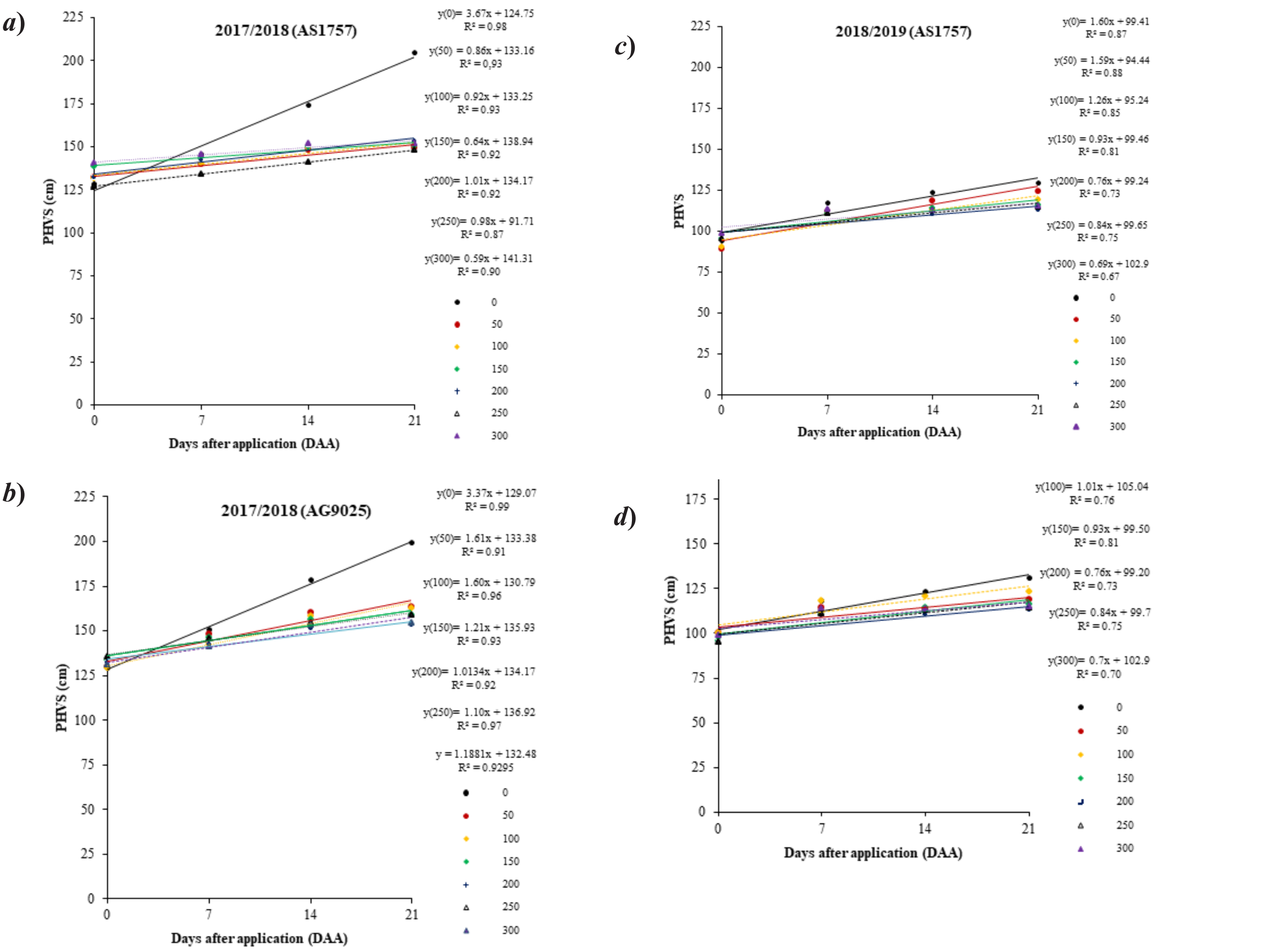

Fig. 2. Effect of growing season for plant height in the vegetative stage (PHVS) of two maize hybrids, in response to seven prohexadione doses and days after application (DAA)

Table 3. Analysis of variance (MS – Mean Square) and significance for NDVI; SPAD chlorophyll index; plant height (PH); ear insertion height (EIH); stem diameter (SD); number of leaves (NL) per plant; leaf area (LA); tassel length (TL); peduncle length (PDL); kernel row number (KRN) per ear and biological yield (BY) of two maize hybrids submitted to increasing doses of prohexadione calcium and as a function of growing seasons (2017/2018 and 2018/2019)

SOV	DF	NDVI	SPAD	PH	SIH	SD	NL	LA	TL	PDL	KRN	BY
				(cm)	(cm)	(mm)	(n°)	(cm^2 pl^{-1})	(cm)	(cm)	(n°)	(g pl^{-1})
MS – $AS1757_{VTPRO3}$												
Block	5	0.05^{ns}	12.28^{ns}	160.50^{ns}	108.16^{ns}	1.71*	0.56^{ns}	156575.12**	7.40**	7.65^{ns}	1.14^{ns}	41.21^{ns}
Year (A)	1	0.28**	18.76^{ns}	4040.74**	9345.19**	908.69**	15.42**	316470.36*	394.33**	76.57**	119.04**	28064.2**
Doses (D)	6	0.14**	63.59**	15505.55**	1480.08**	0.57^{ns}	0.24^{ns}	40985.18^{ns}	28.74**	355.87**	3.65*	3.85**
A x D	6	0.07*	12.05^{ns}	1929.57**	207.32**	2.71^{ns}	0.37^{ns}	14866.06^{ns}	8.78**	8.48^{ns}	1.71^{ns}	1.028^{ns}
Error	65	0.03	11.44	177.69	48.98	0.68	0.29	47470.13	2.22	7.34	1.59	39.89
Overall Mean	-	6.90	28.47	131.73	62.66	14.48	16.54	1092.39	23.07	11.38	13.42	48.86
CV (%)	-	2.64	11.88	10.12	11.17	5.71	3.25	19.94	6.46	23.8	9.4	12.93
MS – $AG9025_{VTPRO3}$												
Block	5	0.44**	23.5^{ns}	404.63*	204.84**	2.46^{ns}	0.32^{ns}	317431.6**	0.47^{ns}	8.61^{ns}	0.45^{ns}	237.5^{ns}
Year (A)	1	0.01^{ns}	265**	9804.24**	14339.36**	1321.7**	20.01**	216964.3*	277.95**	5.00^{ns}	68.76**	53128.8**
Doses (D)	6	0.11^{ns}	18.5^{ns}	10754.89**	1238.03**	0.31^{ns}	0.15^{ns}	82572.4*	69.74**	236.70**	1.30^{ns}	92.49^{ns}
A x D	6	0.17^{ns}	22^{ns}	568.94**	122.76*	2.73^{ns}	0.37^{ns}	8129.0^{ns}	2.87^{ns}	2.21^{ns}	2.31^{ns}	17.74^{ns}
Error	65	0.09	12.58	128.90	46.56	1.29	0.31	31949.5	3.95	6.09	1.54	133.12
Overall Mean	–	6.81	29.44	130.88	63.86	15.07	16.01	1068.38	24.42	11.00	11.71	54.11
CV (%)	–	4.41	12.05	8.67	10.69	7.55	3.48	16.73	8.14	22.43	10.61	21.32

** e * = differ statistically according to the F-test ($p < 0.01$) and ($p < 0.05$), respectively; ns: not significant; SOV = sources of variation; DF = degrees of freedom; CV% = coefficient of variation

ble 2). The PHVS-variable exhibited distinct linear growth as a function of days after PGR application in the different growing seasons for the different experiments but a distinct slope. Restrictive vegetative growth was observed after the application of 50 g ai ha^{-1} of prohexadione. Greater intensity was obtained with 300 g ai ha^{-1} of prohexadione, which produced the lowest growth rates for hybrids $AS1757_{VTPRO3}$ (0.59 and 0.69 $cm.day^{-1}$) and $AG9025_{VTPRO3}$ (1.18 and 0.69 $cm.day^{-1}$) in 2017/2018 and 2018/2019, respectively (Figure 2). Applying prohexadione to maize plants in the 2017/2018 growing season was more effective at restricting vegetative growth in $AS1757_{VTPRO3}$ when compared to the growth rates of $AG9025_{VTPRO3}$. However, there was no difference between hybrids in 2018/2019 (Figure 2a and 2b).

There were significant interaction effects between growing season (A) and prohexadione doses (D), and the primary factors for plant height and ear insertion height in both hybrids. Thus, the hybrids displayed distinct PH and EIH values in the different years tested. NDVI and tassel length also demonstrated interaction (AxD) only in hybrid $AS1757_{VTPRO3}$. Regarding the simple effects of PGR doses, differences were observed in the SPAD chlorophyll index, peduncle length (PDL), kernel row number (KRN) and biological yield (BY) for $AS1757_{VTPRO3}$. For hybrid $AG9025_{VTPRO3}$, leaf area (LA), tassel length (TL) and peduncle length (PDL) differed in terms of the simple effect of prohexadione doses (Table 3).

For hybrid $AS1757_{VTPRO3}$ and $AG9025_{VTPRO3}$, PH in growing season 2017/2018 obtained a quadratic response to the increase in PGR dose. The lowest plant height was found for the prohexadione dose of 211 g ai ha^{-1} (minimum height = -b/2a = 106.8 cm) for $AS1757_{VTPRO3}$ but for $AG9025_{VTPRO3}$ obtaining a minimum (116 cm) at a dose of 216 g ai ha^{-1}. However, the 2018/2019 growing season showed a linear decline bouth hybrids with increasing prohexadione doses, for $AS1757_{VTPRO3}$ decline of 3 cm every 10 g ai ha^{-1} and $AG9025_{VTPRO3}$ decreasing 2 cm for each 10 g ai ha^{-1} (Figure3).

The experiment with hybrid $AS1757_{VTPRO3}$ showed a quadratic response for EIH in the first growing season, producing a lower value (63.6 cm) with 195.5 g ai ha^{-1}. The second year exhibited a decreasing linear response, with a 1 cm decline for each g ai ha^{-1} (Figure3c). Similar results were found for $AG9025_{VTPRO3}$ (quadratic effect), with a minimum EIH (69.8 cm) at a dose of 216.7 g ai ha^{-1} in 2017/2018 and a linear response to a higher dose for the 2018/2019 growing season of around 0.7 cm for each 10 g ai ha^{-1} (Figure 3d).

EIH reductions were less marked in relation to PH in both hybrids, given that prohexadione was applied in pheno-

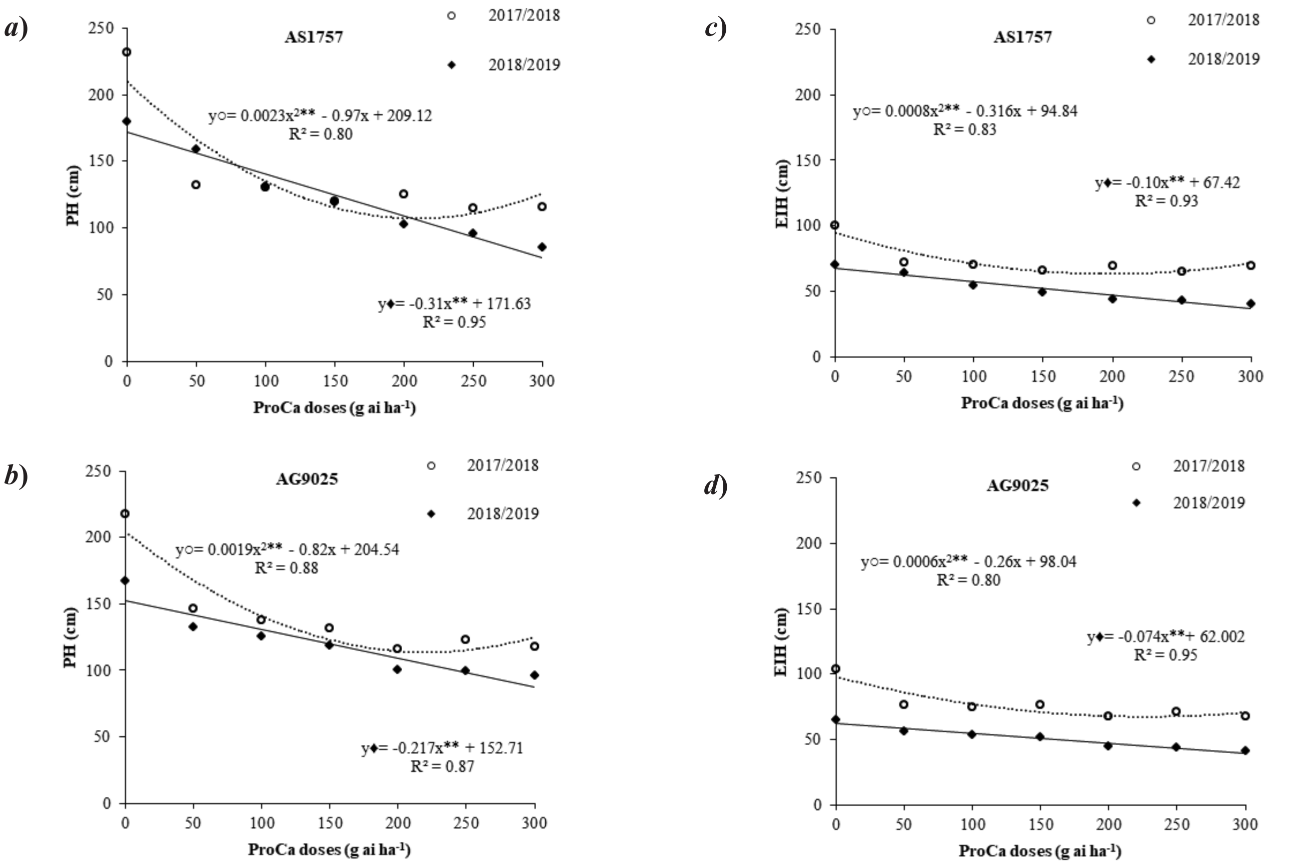

Fig. 3. Plant height (PH) and ear insertion height (EIH) of two maize hybrids, in response to seven prohexadione doses and two growing seasons

logical stage V7, and was efficient at restricting growth in the later phases of vegetative development. Considering PH and EIH together, the internodes located above the ear were the most responsive to the action of prohexadione, but smaller due to the doses of the plant growth regulator.

With respect to the performance of hybrid AS1757$_{VTPRO3}$ in NDVI, a quadratic response was obtained in 2017/2018, with the maximum point (-b/2a = 7.15) reached at 205 g ai ha^{-1} of prohexadione calcium. In the second growing season, there was a linear increase with the use of higher prohexadione doses (Figure 4a). The NDVI of hybrid AG9025$_{VTPRO3}$ was not influenced by the interaction between factors A x D and the primary factors isolated for this variable (mean of 6.81) (Table 3 and Figure 4b). The restricted plant growth with the use of prohexadione doses increased the vegetative indices of hybrid AS1757$_{VTPRO3}$.

Increasing prohexadione doses led to a quadratic response in the SPAD chlorophyll index for the early-maturing hybrid, which displayed maximum efficiency (30.2) at a dose of 191.5 g ai ha^{-1}, with no statistical difference in growing seasons (Figure 4c). Growing season exerted the only effect on AG9025$_{VTPRO3}$, exhibiting a higher mean (31.20) in 2017/2018 than 2018/2019 (27.6) (Figure 4d). The prohexadione calcium in this study, lead in to the increase in chlorophyll per unit of leaf tissue area and/or volume, since the rise in PGR dose reduced biological yield but did not affect the leaf area of hybrid AS1757$_{VTPRO3}$ (Figure 4a and 4c x 6e) and AG9025$_{VTPRO3}$ (Figure 4b and 4d x 6f). Possibly due a better nitrogen distribution, retention and metabolism. In relation to tassel length (TL), there was a quadratic response for hybrid AS1757$_{VTPRO3}$, with a minimum of 23.6 cm at 155 g ai ha^{-1} and 20.1 cm at 222 g ai ha^{-1} of prohexadione in the first and second season, respectively (Figure 5a). Similar behavior was for AG9025$_{VTPRO3}$, quadratic adjustment, minimum 11.1 cm at the dose of 413 g ai ha^{-1} (Figure 5b).

Peduncle length (PDL) obtained a quadratic response with increasing doses of prohexadione for both hybrids, with

Table 4. Stem diameter (SD); number of leaves (NL); leaf area (LA); peduncle length (PDL); kernel row number (KRN) per plant and biological yield (BY) of two maize hybrids for two growing seasons, in average of seven doses of prohexadione calcium

Growing season	SD	NL	LA	PDL	KRN	BY
	(mm)	(n° pl^{-1})	(cm^2 pl^{-1})	(cm)	(n°)	(g pl^{-1})
	AS1757$_{VTPRO3}$					
2017/2018	17.17 a	16.97 a	1153.77 a	10.42 b	14.61 a	67.14 a
2018/2019	11.19 b	16.11 b	1031.01 b	12.33 a	12.23 b	30.58 b
CV (%)	5.71	3.25	19.94	23.8	9.4	12.93
LSD	0.36	0.23	94.95	1.18	0.55	2.75
	AG9025$_{VTPRO3}$					
2017/2018	19.40 a	16.50 a	1119.20 a	26.24 a	12.61 a	79.26 a
2018/2019	11.10 b	15.52 b	1017.50 b	22.60 b	10.80 b	28.96 b
CV (%)	7.55	3.48	16.73	8.14	10.61	21.32
LSD	0.49	0.24	77.89	0.8	0.54	5.02

Means followed by the same letter in the column do not differ statistically, according to the Tukey test ($p < 0.05$). LSD: least significant difference. CV: coefficient of variation

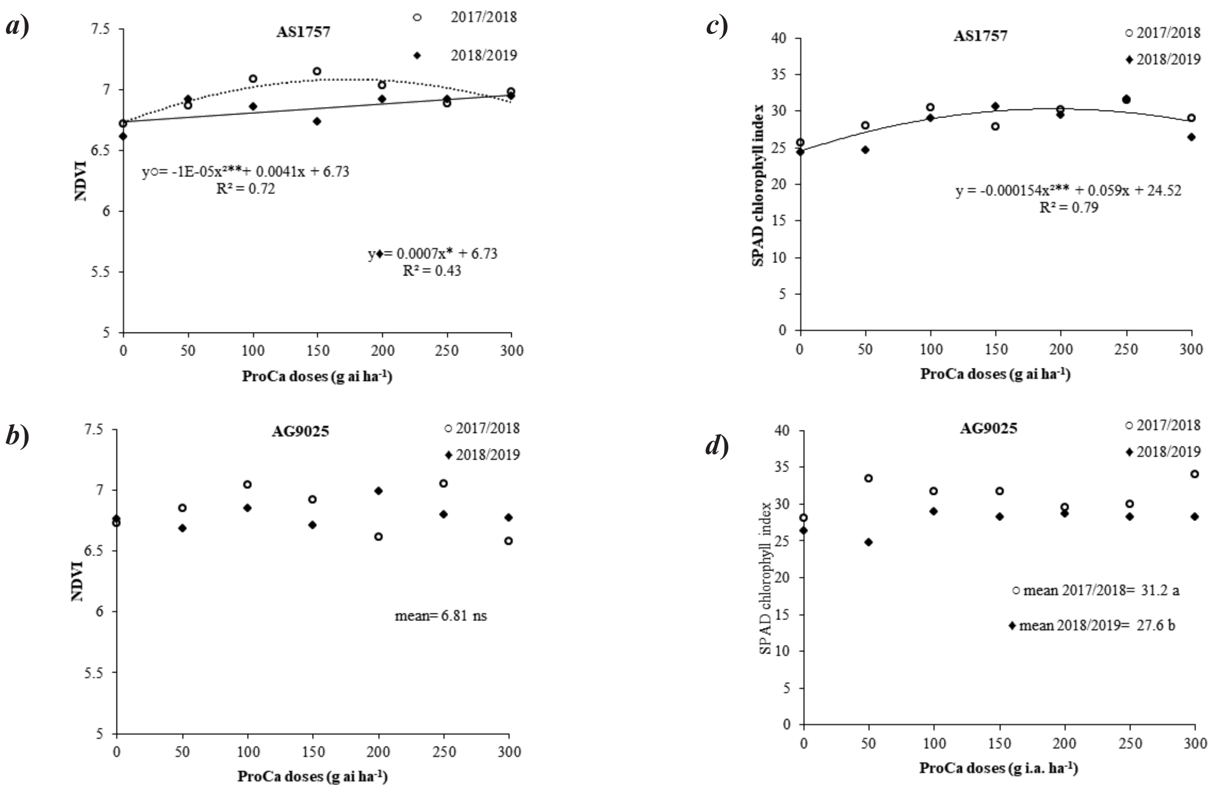

Fig. 4. The normalized difference vegetation index (NDVI) of the two maize hybrids: *a*) as a response to growing season and prohexadione doses in hybrid AS1757$_{VTPRO3}$; *b*) average of two growing seasons and seven prohexadione doses in hybrid AG9025$_{VTPRO3}$; *c*) SPAD chlorophyll index in response to prohexadione doses in the average of two growing seasons for hybrid AS1757$_{VTPRO3}$ and *d*) average of two growing seasons and seven prohexadione doses for hybrid AG9025$_{VTPRO3}$

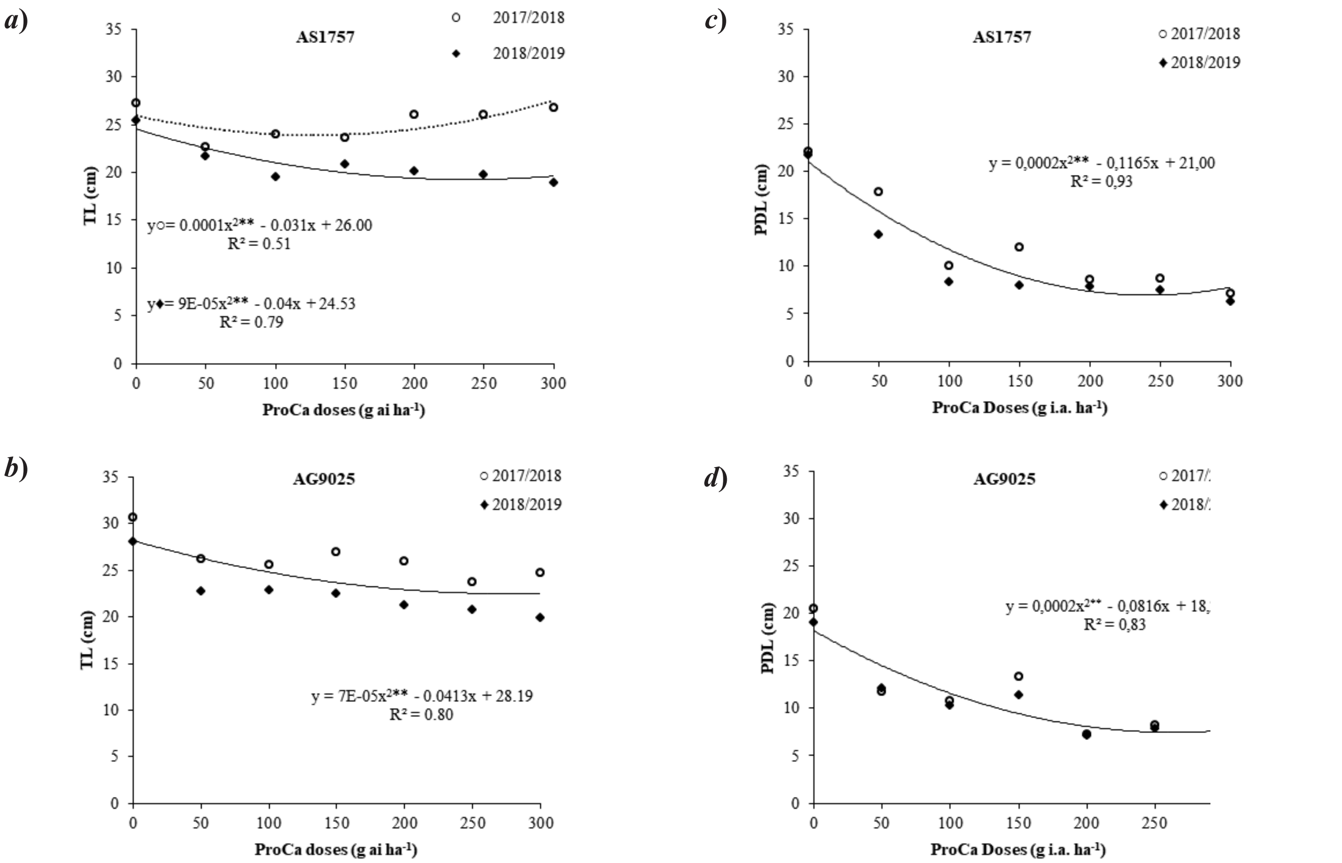

Fig. 5. Tassel length (TL) of two maize hybrids: *a*) in response to growing season and prohexadione doses in hybrid AS1757$_{VTPRO3}$ and *b*) in response to prohexadione doses in the average of two growing seasons for hybrid AG9025$_{VTPRO3}$. Peduncle length (PDL) as a response to seven prohexadione doses in the average of two growing seasons for hybrids *a*) AS1757$_{VTPRO3}$ and *b*) AG9025$_{VTPRO3}$

minimums of 4 cm at 291.2 g ai ha^{-1} (Figure 5c) and 8.2 cm at 202.5 g ai ha^{-1} (Figure 5d) for AS1757$_{VTPRO3}$ and AG9025$_{VTPRO3}$, respectively. This variable is associated with the ability of the PGR to reduce the morphometric traits of the maize plant. The effect of PGR on maize crops occurs in the upper internodes of the stem, such as those located above the ear, TL and PDL. In this study, when TL and PDL are taken together, prohexadione caused a maximum reduction of 23 cm in hybrid AS1757$_{VTPRO3}$, and 18.8 cm in AG9025$_{VTPRO3}$.

The prohexadione dose above 100 g ai ha^{-1} lowered the KRN per ear (Figure 6a) in hybrid AS1757$_{VTPRO3}$. The KRN response to prohexadione may is linked to the changes in plant morphology caused by the molecule, specifically the application stage and effects of restricting longitudinal plant growth. Maintaining photosynthetically active (green) leaves in the reproductive phase of plants and changes in leaf size as a function of PGR application caused a linear increase in leaf area with a rise in prohexadione doses for hybrid AG9025$_{VTPRO3}$ (Figure 6d) and a non-statistical difference in this variable with prohexadione doses for AS1757$_{VTPRO3}$ (Figure 6c). Biological yield declined linearly with a rise in prohexadione doses for AS1757$_{VTPRO3}$ (Figure 6e; decrease of 0.26 g for each 10 g ai ha^{-1}), confirming vegetative growth restriction in the two growing seasons, primarily in plant height (Figure 6a).

Stem diameter (SD) was not affected by prohexadione doses in the two hybrids tested, but there was a difference between growing seasons (Table 1 and Figure 4). The variables NL, LA, PDL, NRK and BY were also affected by growing season in both experiments. In the first study year, the experiments obtained higher average values, except for PDL in AS1757$_{VTPRO3}$ (Table 1). These responses may be influenced by environmental factors such as greenhouse humidity, temperature and light, as well as the endogenous factors of each hybrid assessed.

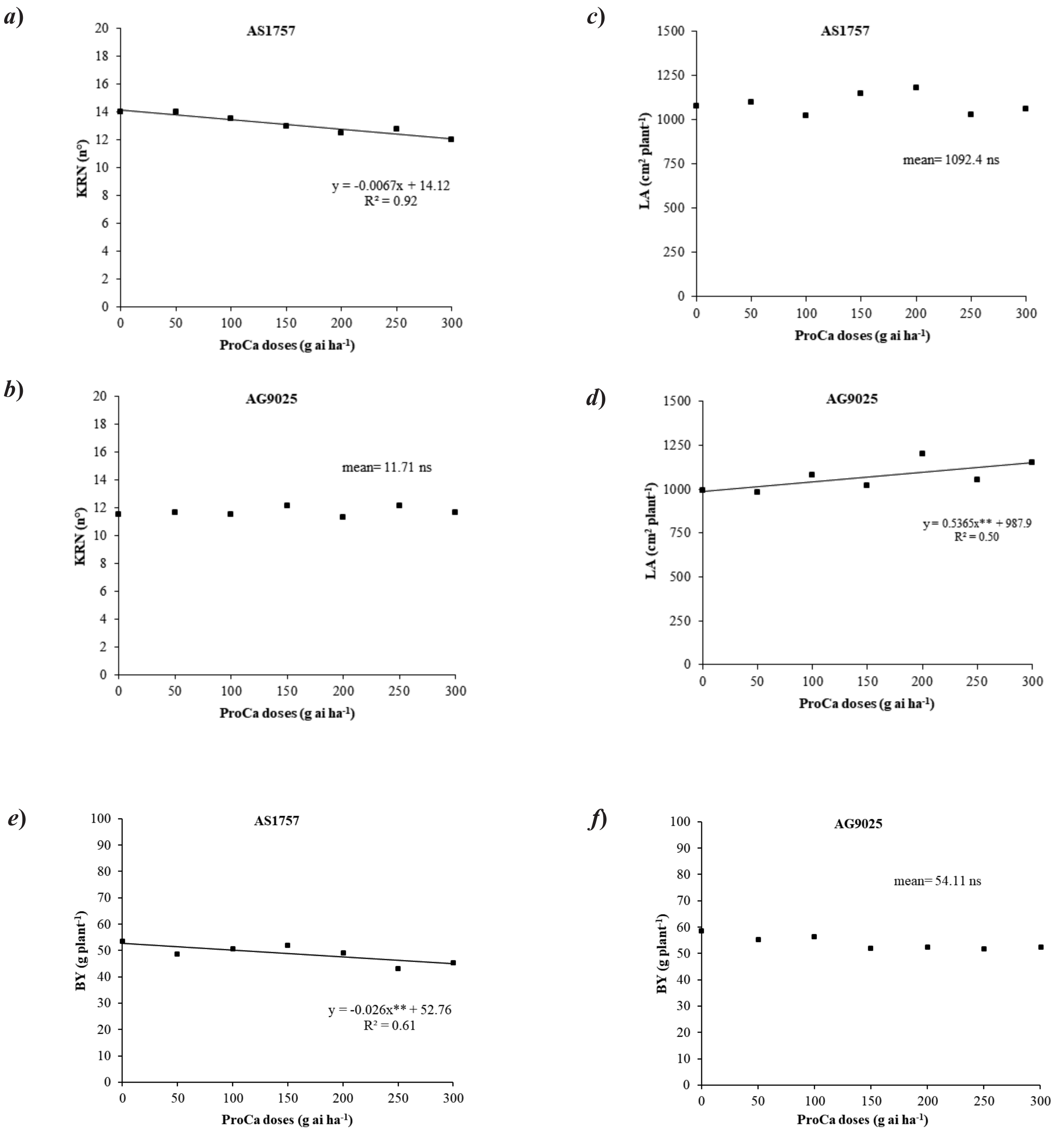

Fig. 6. Kernel row number (KRN) per ear in two maize hybrids in reponse to seven prohexadione doses, in the average of two growing seasons in hybrid AS1757$_{VTPRO3}$ (*a*) and in hybrid AG9025$_{VTPRO3}$ (*b*); leaf area (LA) in two maize hybrids in response to seven prohexadione doses, in the average of two growing seasons in hybrid AS1757$_{VTPRO3}$ (*c*) and in hybrid AG9025$_{VTPRO3}$ (*d*); biological yield (BY) per plant of two maize hybrids inresponse to seven prohexadione doses, in the average of two growing seasons in hybrid AS1757$_{VTPRO3}$ (*e*) and in hybrid AG9025$_{VTPRO3}$ (*f*)

Discussion

The short-time plant height (PHVS) in response to prohexadione doses was observed as function of season and hybrid, with different growth intensities like a response previously reported in studies with the PGR trinexapac-ethyl in white-oat (Hawerroth et al., 2015) and wheat crops (Espindula et al., 2010). Like that observed with trinexapac-ethyl in the maize crop under study in a greenhouse (Pricinotto et al., 2015; Mendes Fagherazzi et al., 2018) the use of higher doses of prohexadione caused less longitudinal growth, regardless of growing season and hybrid.

This performance demonstrates the efficiency of the prohexadione in acting on the meristems of maize and impeding the complete internode elongation, which restricted plant growth. Prohexadione impedes complete cell elongation via GA_{20} 3β hydroxylase enzyme inhibition at the end of the gibberellin biosynthesis route, thereby reducing the endogenous levels of active gibberellin (GA_1) and increasing its inactive precursor (GA_{20}) (Davies, 2010). The decline in active gibberellic acid (GA_1) levels inhibits plant growth (Rademacher, 2015).

In a study of field-grown rice (*Oryza sativa*) (Na et al., 2011) and flax (*Linum usitatissimum* L.) cultivated in a greenhouse (Kim et al., 2018) applying prohexadione was effective in restricting the longitudinal growth of the main plant stem. On the other hand, in a field study with a prohexadione dose of 63 g ai ha^{-1}, Spitzer et al. (2015) found no statistical difference in maize growth restriction. A discrepancy between the results of Spitzer et al. (2015) and those obtained here was also observed in ear insertion height.

Leolato et al. (2017) and Mendes Fagherazzi et al. (2018) observed a decline in ear insertion height after application of the trinexapac-ethyl. The authors report that plants with smaller PH and EIH would exhibit agronomic advantages, since their center of gravity is more balanced and the level of stem lodging and rupture is lower, favoring nutrient absorption and assimilate synthesis for grain production.

Ozbay and Ergun (2015) also observed an increase in eggplant leaf chlorophyll content (*Solanum melongena* L.) with a rise in prohexadione dose (0, 50, 100 and 150 mg L^{-1}). The effect of trinexapac-ethyl on maize (Pricinotto et al., 2015) and wheat crops (Espindula et al., 2009), extend to prohexadione in this study, is related to the increase in chlorophyll per unit of leaf tissue area and/or volume, since the rise in PGR dose reduced biological yield but did not affect the leaf area as observed on hybrid $AS1757_{VTPRO3}$ (Figure 6c and 6d versus 6e and 6f). Zagonel & Ferreira (2013) assessed two maize hybrids (Status TL and Maximus TLTG) as a function of trinexapac-ethyl (0, 187.5, 375 and 562.5 g ai ha^{-1}), and observed that the Maximus hybrid exhibited quadratic behavior, reaching a maximum leaf area with 220 g ha^{-1}. However, Pricinotto et al. (2015) found a linear reduction in maize leaf area with an increase in trinexapac-ethyl dose (0, 125, 250 and 375 g ai ha^{-1}).

The restricted plant growth with the use of prohexadione doses may increased the vegetative indices. According to Vian et al. (2018) the NDVI can be used to identify the spatial variation of shoot biomass production in a crop and monitor leaf chlorophyll content. Variability caused by population and plant growth alterations determines distinct productive potentials in the maize crop. Espindula et al. (2010) also observed a quadratic response for this variable, obtaining a minimum value with 1183 g ai ha^{-1} of chlormequat chloride in a wheat crop.

The differences in internodes length are related to the application stage and number of times the product was applied. Leolato et al. (2017) observed a decrease of 8 cm between the main ear to the tip of the tassel with 150 g ai $ha^{-1}$of trinexapac-ethyl, applied in stages V5 plus V10. In contrast to winter cereals, the effect of plant growth regulators on maize crops occurs in the upper internodes of the stem, such as those located above the ear, TL and PDL. According to Dourado Neto et al. (2014) KRN is determined from stage V6 to V12, depending primarily on the nutritional conditions, water availability and in the case of this study, genotype. This differs from the results of Pricinotto et al. (2015) that used trinexapac-ethyl and found no significant changes in KRN. These opposite results may due use different PGR, doses, plant growth stage of application, plant species and environments (different season) particularly as observed on ours assay conditions, in first season when temperature trend an increase compared to a decrease trend in second season (Figure 1).

Espindula et al. (2009) observed a linear reduction in biological yield (BY) with doses of chlormequat chloride (0, 500, 1000 and 1500 g ha^{-1}) and trinexapac-ethyl (0, 62.5, 125.5 and 187.5 g ai ha^{-1}) in a wheat crop. According to the authors, a decrease in BY is associated with a decline in grain yield, and a rise in trinexapac-ethyl doses. This, in turn, is related to the lower kernel row number per ear and BY found in hybrid $AS1757_{VTPRO3}$ (Figure 6a and Figure 6e). The BY of hybrid $AG9025_{VTPRO3}$ remained constant with an increase in prohexadione doses (Figure 6f). Zagonel & Ferreira (2013) also reported no statistical difference in kernel row number per ear after applying trinexapac-ethyl to Status TL and Maximus maize hybrids.

The SD no response to PGR were results anothers works, like obtained by Pinheiro et al. (2018) with prohexadione doses (0, 100 and 200 g ai ha^{-1}) in maize hybrid P30F53YH, showing no significant difference between treatments. Although SD is important in raising resistance to lodging and plant rupture, it does not always change in wheat crops after PGR application (Zagonel & Ferreira, 2013). According to the Ministry of Agriculture, Livestock and Supply (Brasil, 2019) the prohexadione (Commercial product Viviful® WG) reduces the stem length of oat, rye, barley, wheat and triticale plants, making them compact, corroborating the results obtained here for maize. However, it differs in terms of strengthening the internodes of these crops when compared to maize plants.

Similar results were obtained by Mendes Fagherazzi et al. (2018) testing maize genotypes under greenhouse conditions, in response to trinexapac-ethyl applied in different phenological stages over two growing seasons. They found different behavior between the mean values of the two seasons for plant and ear insertion height, stem diameter, number of leaves and biological yield. According to the authors, plant growth and development are regulated by intrinsic and external factors, for exemple temperature trend or air humidity (Figure 1a and 1b). The factors inherent to the intrinsic to plant are controlled at the cellular and molecular level, as well as by plant hormones, whose function is to regulate the organism. These plant hormones are triggered by external stimuli and, as such, all plants organisms are informed of the situation of other parts by synthesis or changes in concentration of one or more hormone.

Conclusions

Applying prohexadione calcium in the phenological stage V7, regardless of dose, reduces vegetative growth, restriction in plant height the hybrids 47% for $AS1757_{VTPRO3}$ and 57% for $AG9025_{VTPRO3}$.

Applying prohexadione reduced the phytometric characters ear insertion height, length of the tassel and peduncle, biological yield and kernel row number per ear, however, the responses depend on the genotype and the year of application.

Leaf architecture changed in hybrid $AG9025_{VTPRO3}$, rising up to 210 cm^2 per plant with an increase in prohexadionedose. With respect to the increase in optical sensor values, the NDVIand SPAD chlorophyll indices by 6 and 26%, respectively, with a rise inprohexadione doses only in hybrid $AS1757_{VTPRO3}$.

The early maturing group hybrid ($AS1757_{VTPRO3}$) was more responsive to prohexadione doses when compared to its super-early ($AG9025_{VTPRO3}$), suggesting that the morphological and physiological parameters of hybrids with latest cycles are more responsive to prohexadione.

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