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Phenology and thermal requirements of the species *Cyperus difformis* L. in southern Brazil

Higor Modesto Garlet Voss, Otávio dos Santos Escobar, Vinícius Severo Trivisiol, Mariane Peripolli*, Maicon Pivetta, Jaíne Rubert, Elisandro Azeredo Nunes and Sylvio Henrique Bidel Dornelles

Programa de Pós-Graduação em Agronomia, Universidade Federal de Santa Maria, Av. Roraima, 1000, 97105-900, Santa Maria, Rio Grande do Sul, Brazil. *Author for correspondence. E-mail: mperipolli@gmail.com

ABSTRACT. *Cyperus difformis* L. is a Cyperaceae, annual, of natural occurrence in marshy environments in southern Brazil, considered a weed in irrigated rice crops. Studies on its development are scarce, especially regarding its cycle and thermal requirements. Therefore, the objective of this work was to determine the base temperature, the thermal sum and the duration of the different sub-periods of the biological cycle of the species. The experiment was carried out at the Campus of the *Universidade Federal de Santa Maria – UFSM*, Santa Maria, Rio Grande do Sul, in four sowing seasons. In a completely delineated design and factorial scheme involving five soil water conditions (water depth and 50% soil water retention capacity [WRC]) from the sowing, water depth and 50% WRC introduced 21 days after emergence and 100% WRC during the whole cycle, with six repetitions each. Each experimental unit consisted of a cultivated in pot plant with 11 L capacity filled with soil. The cycle of the plants was subdivided into the sowing-emergence sub-periods, emergence-emission of the floral tassel and emission of the floral-maturing physiological tassel, being estimated the thermal requirements for the species. The conditions of the soil water condition the base temperature, the sum of the degree-days and the duration of the species cycle. The base temperature varies for each treatment, the sum of degrees-day decreases with the water deficit and the plants under flood accelerate the biological cycle.

Keywords: phenology; biological cycle; adaptation; water conditions.

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Introduction

The species *Cyperus difformis* L. is considered an invasive plant in rice fields in southern Brazil, mainly in the pre-germinated crop system. This system is preferably used to physically control species with a high level of resistance that are established during the cultivation cycle, especially at its beginning.

The species *C. difformis* has the capacity to develop in flooded environments, despite presenting a C3 photosynthetic mechanism, which is not the most efficient, it finds in this environment the best conditions for its development, thus, being possible to produce a large amount of energy and to advance its cycle (Taiz, Zeiger, Møller, & Murphy, 2017) in such a way that species can develop more than one cycle in relation to that of irrigated rice. In addition, herbicide resistance was observed, mainly in the chemical group of inhibitors of the enzyme Acetolactate Synthase (ALS) (Vargas et al., 2016), which is widely used in rice cultivation in southern Brazil.

Although there are several studies about the resistance of *C. difformis* to herbicides (Galon et al., 2008; Dal Magro et al., 2010; Chiapinotto, Schaedler, Fernandes, Andres, & Lamego, 2017), little information is available about its biology and phenology. This occurs for the majority of the cultivation invaders, even for those that promote significant losses in agricultural crops in Brazil. However, it is important to emphasize the work, recently conducted by Carloto (2016), on the physiology and phenology of two species of the genus *Eragrostis*, also considered an invader in the rice culture in the region. This shows the concern of the research area regarding the damages these new invaders are causing to the rice farmers. The knowledge of the phenology and the bioclimatic requirements of these species are important because it allows to predict the probable dates of the occurrence of the different phases of the vegetative and reproductive cycle, enabling the rice farmer to make the most appropriate management of the culture tracts in the crop.

Meteorological factors are one of the main determinants of plant phenology and, among them, soil and air temperature. Plants require the accumulation of a certain amount of heat, commonly expressed by the sum of degree-days, which represents the thermal sum above the minimum base temperature for development to complete each physiological sub-period of the life cycle. The degree-day concept assumes the existence of a base temperature, under which plant growth and development are disrupted or greatly reduced (Brunini, Lisbão, Bernar-DI, Fornasier, & Pedro Júnior, 1976; Kishimoto-Yamada & Itioka, 2015).

In the case of the *C. difformis* species, no studies on its phenophase and thermal requirements are known. Thus, the objective in this work was to determine the thermal requirements of the different sub-periods of the plant cycle of *C. difformis* species.

Material and methods

Four experiments were performed at different times, with sowing intervals between the dates of approximately 23 days. Seeds were harvested on 10/25/2016, 11/14/2016, 10/12/2016 and 05/01/2017, respectively, at a predetermined time of 15 hours. The experiments were carried out inside a plastic greenhouse with dimensions of 6x20 and 5 m hight, located in the Department of Biology of the *Universidade Federal de Santa Maria - UFSM* (latitude: 29° 42' 52.3" S, longitude: 53° 43' 8.01" W, altitude: 102 m), Santa Maria, state of Rio Grande do Sul.

For the experiments, *Cyperus difformis* accessions were collected in an irrigated rice cultivated area in Meleiro, state of Santa Catarina (latitude: 28º 53' 1,966" S; longitude: 49º 33' 8,376" W and altitude: 12 m). After the physiological maturation, 10 g of seeds of a single plant of the ten of each sampling area, randomly chosen, were collected for the execution of the experiment. The trial design was completely randomized, with a factorial arrangement: five soil water conditions, with six repetitions. The water conditions tested were: 100% of soil water retention capacity (WRC), water depth from sowing (5 cm), 50% of WRC from sowing, water depth introduced 21 days after sowing (AED) and 50% of the WRC introduced at the 21 DAE, and the last two conditions, until the moment of the introduction of the treatments, remained on the 100% WRC water condition. The introduction of the water treatments of water depth and 50% WRC after the 21 DAE were performed on 11/24/2016, 08/12/2016, 08/01/2017 and 04/02/2017, respectively.

Phenological observations were made daily throughout the plant cycle, characterizing the sowing-emergence sub-periods (SOW-EME), emergence-emission of the floral tassel (EME-EMI) and emission of the floral tassel-physiological maturity (EMI-MAT). The beginning and end of these sub-periods were defined, respectively, from sowing to finishing in the emergence of the first 15 seedlings (50% of the total) of the six repetitions, from the emergence of the seedlings to the emission visualization, the floral tassel (> 2 cm) of the floral tassel emission (> 2 cm) until the physiological maturation of the plants (seeds presenting a brown color).

The experimental units were constituted by flexible plastic vessels (Nutriplant) of 11 L of volume, filled with 12 kg of soil classified as *Haplic Acrisol*, horizon A (Streck et al., 2008), which allowed a spare of 5 cm for the treatments that received the water depth. The soil was sieved and corrected according to the chemical analysis, following the indications for irrigated rice crops (Silva, Gatiboni, Anguinoni, & Souza, 2016). The results of soil chemical and physical analysis were: pH in water (1: 1) = 4.25; organic matter = 2.3%; P = 3.3 mg dm^{-3}; K = 0.119; Ca = 0.3; Mg = 0.1; H + Al = 7.3; and Effective CTC = 2.7 cmolc dm^{-3}; Saturation Bases = 7%; total sand = 412.28 g kg^{-1}; silt = 419.72 g kg^{-1}; and clay: 168 g kg^{-1}.

The determination of the water retention capacity (WRC) of the sieved soil was performed using the weighing method, in which the soil was dried in an oven at 70°C, being weighed in a precision scale of 0.01 g per hour to constant mass. After the drying process, 3 kg of dry soil were placed in a known mass vessel, containing holes in the base, which was soaked to saturation and subsequently subjected to constant mass drainage. Considering that the specific mass of the water is 1000 kg m^{-3} or 1 kg L^{-1}, 100% of the water retention capacity of the soil was determined by the difference in weight of the pot with dry soil with the one in which it left drain the water.

The following formulas were used to determine the water content of the different treatments (50% and 100% of the WRC), according Equation 1 and 2:

$$WV100\% = (WVWRC - WVdry).1 + WV\ dry, \quad (1)$$

$$WV50\% = (WVWRC - WVdry).0,5 + WV\ dry, \quad (2)$$

WVn% is the pot weight for each treatment; WVWRC is the mass of the pot in the water retention capacity of the soil; WVdry is the mass of the pot filled with dry soil. A plastic mesh splint of 0.2 mm was used to cover the holes in the bottom of the pot to avoid possible loss of the sieved soil and plastic bag coating the interior of the pots that received the water depth.

The different irrigations were conducted daily. For maintenance, each pot was weighed using an ACS System brand electronic scale with a precision of 5 g, adding water until reaching the predetermined total mass (pot + dry soil + volume of water to reach 100 and 50% of the WRC).

Soil temperatures were obtained by means of a T-sensor installed 0.5 cm deep and recorded using the Datatlogger Fourtec DaqPRO™ 5300 equipment, and automatic data collection was performed every 5 min. in the five soil water conditions of five reading channels. Soil temperatures were used to determine the thermal requirements of the sub-period corresponding to sowing-emergence of the seedlings. In order to determine the thermal requirements of the emergence-emission sub-periods of the floral-physiological maturity tassel, data were collected from the air temperature, recorded by the Akron KR420 Datalogger, properly calibrated, with a recording frequency of 10 min., the equipment was positioned in a weather shelter at a height of 1.5 m from the ground.

The base-temperature for the initial, vegetative and reproductive sub-periods was calculated using the least variability method (Arnold, 1959; Brunini et al., 1976).

In the lower variability method, the values of temperatures to be used in the calculation of the thermal sums were chosen a priori, and then the standard deviation (Sdd) was determined, in degrees-day. The base temperature of the sub-period was considered to be the one with the lowest standard deviation (Sd), in days.

The lowest standard deviation (Sd) was calculated using the Equation 3:

$$Sd = \frac{Sdd}{(Xt - Tb)} \quad (3)$$

where:

Xt was the average temperature for the series of experiments performed and Tb, base temperatures tested, which were: 1 in 1 degree Celsius, 0 to 30°C.

After the physiological maturation of the plants, total dry matter evaluations were performed, and the root and aerial parts of the plant were collected and dried in an oven at 70ºC until reaching a constant mass. For the total dry matter results, they were tested for the normality of their distribution through the Shapiro-Wilk test and the homogeneity of the variances through the Bartlett test, with the aid of the Action Program (ESTATCAMP, 2011). Subsequently, the analysis of variance (ANOVA) and the Scott-Knott's test for grouping of averages were performed in a 5% probability of error ($p < 0.05$) using the statistical program Sisvar® 5.3 (Ferreira, 2011).

Results and discussion

In the treatment with 50% of the water retention capacity (WRC) of the soil from the sowing the seeds did not emerge, being verified that the species *Cyperus difformis*, under conditions of water deficit from the sowing, is not able to emerge. Thus, the species is not adaptable to emergence in drier environments, and it may be that information, pertinent to rice farmers, since through physical management such as drainage of the areas in the off-season and pre-sowing, would significantly reduce the infestation of the invasive plant and, thus, the initial competition with the irrigated rice in its initial phase of development.

There were differences in the thermal requirements of the plants between the different sub-periods and for the same sub-period, when submitted to different soil water conditions. This was observed for the base-temperature (Figure 1) and the sum of degrees-days (Table 1), as well as the duration of the sub-periods (Figure 2).

The temperature-base of growth in the sowing-emergence sub-period (SOW-EME), Figure 1, was equal to 7 and 15°C and the average duration, Figure 2, of 4.8 and 6.2 days, respectively, for sowing seedlings in soil treatment with 100% soil water retention capacity (WRC) and 5.0 cm of water depth since sowing. The average temperatures in the sub-periods were 22.2; 22.6; 25.7; 25.8°C, average equal to 24.1 and 22.9; 24.9; 26.8; 27.3°C, average equal to 25.4°C. The thermal sums in day-degrees of 39.3; 48.3; 59.1 and 48.9°C, average equal to 48.9 and 106.7; 93.8; 112.1 and 113.1°C, average of 106.4°C, respectively, for soil with 100% WRC and 5 cm of water depth since sowing.

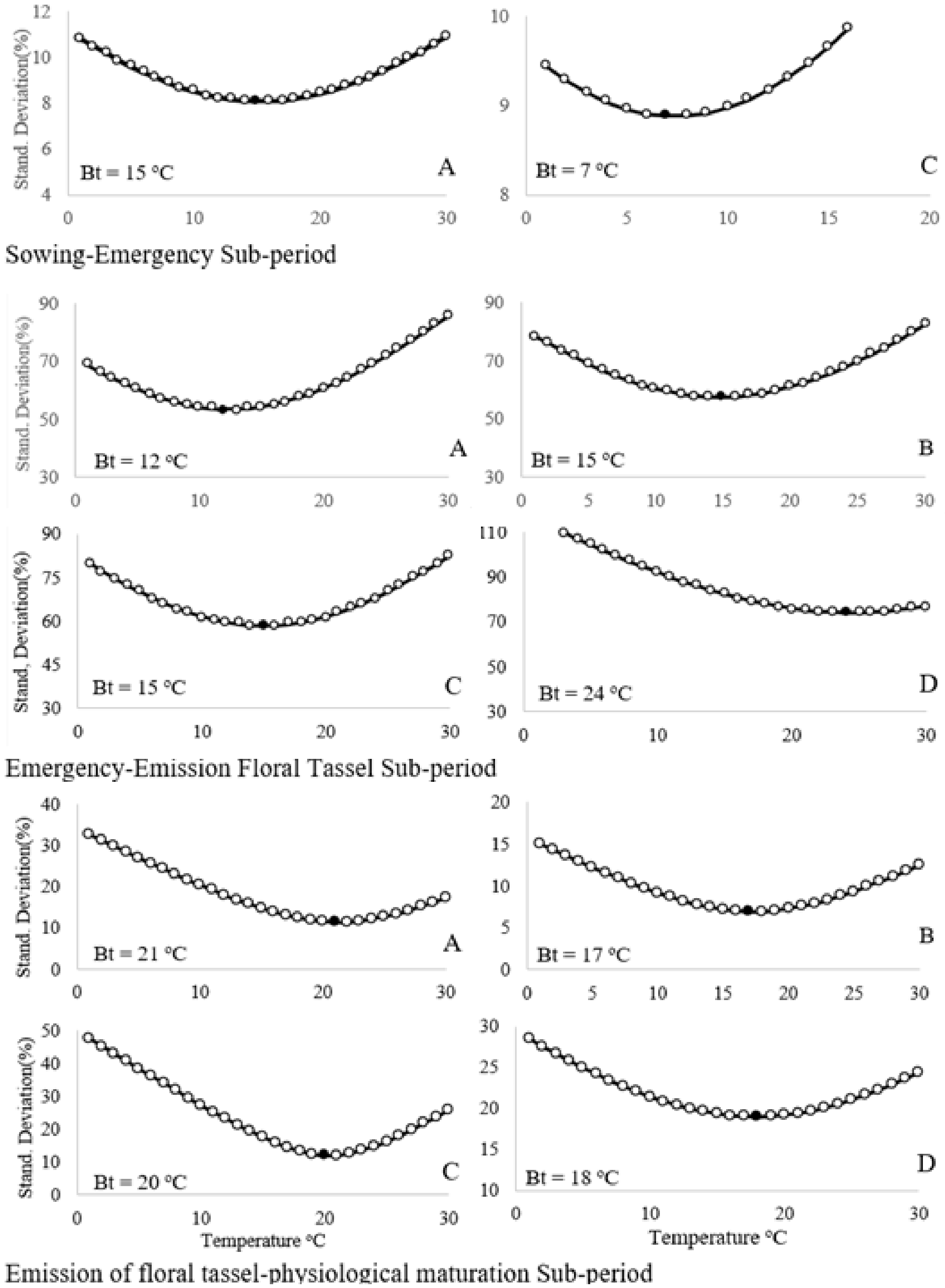

Figure 1. Base-temperature growth calculated by the method of lowest standard deviation in days for the sowing-emergence sub-periods, emergence-emission of the floral tassel, and floral-maturation emission of the species *Cyperus difformis*, conducted in water conditions in the soil water depth of 5.0 cm from seeding (A) a water depth of 5.0 cm applied 21 days after emergence (B) 100% soil water retention capacity (WRC) (C) and 50% soil water retention capacity (WRC) (D), respectively. Santa Maria, state Rio Grande do Sul, 2018.

Table 1. Average of total dry mass of *Cyperus difformis* plants, under different soil water conditions. Santa Maria, state Rio Grande do Sul, 2018.

Average of dry mass of whole plant (g)			
Water condition			
Water depth (cm) from sowing	Water depth (cm) introduced at 21 DAE	100% of WRC	50% of WRC
112.6 a	90.0 a	58.9 b	23.3 c

Averages not followed by the same letter in the line, differ by Scott-Knott's test, in 5% probability. WRC: soil water retention capacity.

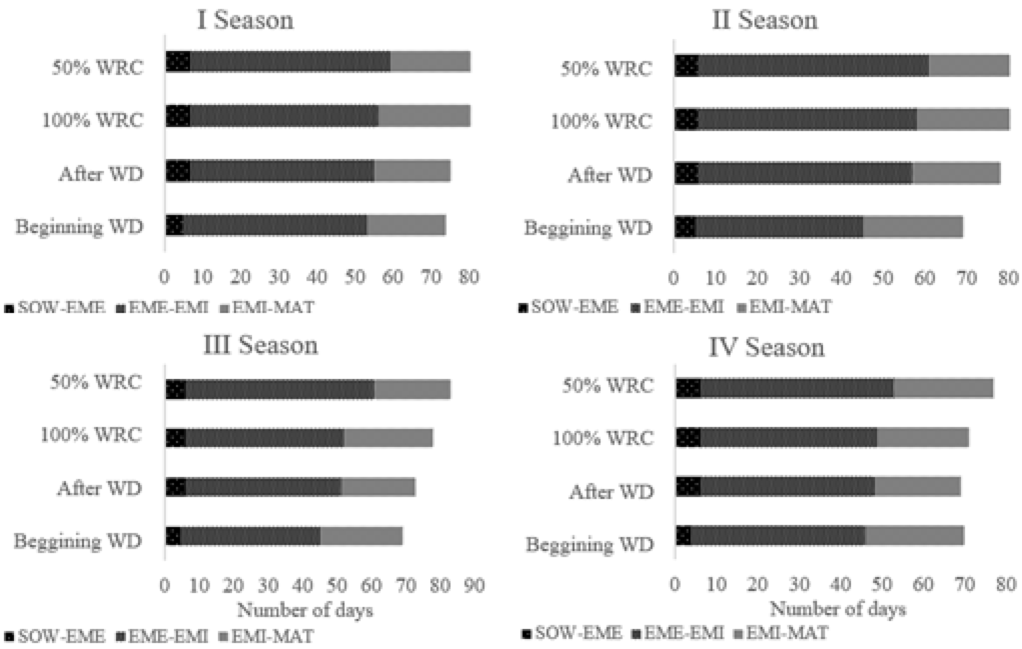

Figure 2. Duration, in days, of phenological subperiods sowing - emergence, emergence - floral tassel emission, floral tassel emission - physiological maturation of plants of the species *Cyperus difformis* submitted to different soil water conditions: soil water depth of 5.0 cm from sowing, 5.0 cm water depth applied 21 days after emergence, 100 soil water retention capacity (WRC) and 50% soil water retention capacity (WRC). Santa Maria, state Rio Grande do Sul, 2018. SOW-EME = sowing - emergence; EME-EMI = emergency - emission of floral tassel; EMI-MAT = Emission of floral tassel - Physiological maturation. WD = Water Depth. Experimental times: I season = 25/10/16; II season = 14/11/16; III season = 10/12/16 and IV season = 05/01/17.

The difference in the base temperature between the treatments for the SOW-EME sub-period was 8.0°C, considered, in this case, very high, which possibly corroborated the difference in the speed of emergency, in the treatments with 5.0 cm of water depth from the sowing and 100% of the WRC, being the emergence of the weed species faster than the irrigated rice. The difference found between treatments of water depth since sowing and 100% of WRC in emergence of seedlings may be related to a number of factors, such as the aquatic habit of the species, where the effect of water pressure on the seed increases its water uptake through the pores and the buffering of the environment by water, significantly increasing the germination speed of *C. difformis* (Derakhshan & Gherekhloo, 2013).

In the emergence-emitting of floral tassel sub-periods (EME-EMI) and floral tassel emission - physiological maturation (EMI-MAT), differences in temperature-base growth values between water conditions were also high (Figure 1) of water deficit (50% of WRC), the plants during the EME-EMI sub-period demonstrated a base-temperature of 10°C above the other water conditions, with the average temperature recorded at different times in this condition only 3.0°C above the base-temperature, resulting in only 32% of the average of the other thermal sums, under conditions without any water restrictions for development (Table 1). In the sowing-emergence sub-period (SEM-EME), the heats of the base growth temperature were not calculated under a condition of 50% of the water retention capacity in the soil due to the lack of conditions for the germination of *Cyperus* seeds, it also points out that, in this subperiod, the 21 days after emergence for the implementation of the water depth were not completed, therefore, both cases were represented by the condition of 100% of the water retention capacity in this subperiod.

Bold points determine the base-temperature for the water conditions, according to the lowest indices of standard deviation found by the least variability method.

In the EMI-MAT sub-period, the base temperature of the plants submitted to 50% WRC water restriction was reduced by 6°C (Figure 1) compared to the EME-EMI sub-period, increasing by 20% the accumulation of the thermal sum of this sub-period, as a way to mitigate energy production to complete the biological cycle and produce seeds. Opposite results were verified mainly for the 5 cm water depth conditions from the beginning and 100% WRC, when in the final sub-period, they increased the base temperature by 9 and 5°C (Figure 1), respectively, in relation to the previous sub-period, resulting in lower accumulations of thermal sum.

In Table 1, the results of the thermal sum or sum of degree-days, of the sub-periods: SOW-EME; EME-EMI; EMI-MAT of the *Cyperus difformis* species submitted to different soil water conditions. It is observed that soil water conditioning interfered in the sum of degree-days (ºC), where, according to the general averages of the treatments under study, there was a decrease in the thermal sum due to the decrease of water content in the soil. For example, the final average of the sum of degree-days in the 50% WRC treatment was 40% lower than in conditions with high soil water content. This result is a consequence of the high values of the base temperature in the condition of 50% WRC of the soil introduced at 21 DAE. This explains the statistically significant difference of the results of total dry matter exposed in Table 1.

In the results of Table 2, it is possible to verify that the greatest accumulation of thermal energy by the plants, in the different soil water conditions, occurred in III experimental period, when it is most sensitive (15/12/16 to 15/02/17) present high daily temperature averages. In the sub-period of the emergency to the emission of the floral tassel, in the four water conditions of the soil and in the four seasons of sowing, there was a greater accumulation of the recorded thermal sum, coinciding with the vegetative period of the plants, when it occurs the need for higher reserves production, a period in which it is highly sensitive to temperature, due to the beginning of the differentiation of the floral beginnings, just as it occurs with rice (Streck et al., 2007).

Figure 2 represented the duration of the sub-periods plants of the species *C. difformis* water subjected to different soil conditions in the four experimental periods. It is observed that the longest sub-period of the plants was the emergence - emission of the floral tassel, with the highest distinction between water conditions of 5 cm of water from sowing and 50% of WRC, and under water deficit, the plants extended their sub-period in 12 days. It is supposed mainly due to the fact that plants with C_3 photosynthetic route, as in the case of *C. difformis* species, under low levels of water in the soil, it triggers defense mechanisms such as stomatal closure, considerably reducing stomatal conductance and, consequently, photosynthesis (Taiz et al., 2017).

According to the values represented in Figure 3, the total duration of the biological cycle of the species *C. difformis* L. suffered interference as a function of soil water conditions: with the presence of a 5 cm water depth from the sowing (natural environment of the species), the cycle of the plants was completed in 70.5 days, prolonging as the water supply was protected, lasting 73.7, 78.2 and 82.2 days in the water table conditions introduced at 21 DAE, 100 and 50% WRC introduced at 21 DAE, respectively.

Due to the results obtained in the present study, especially those obtained for the base-temperature of growth and the speed of emergence of the seeds under water, it is suggested more studies on the subject to be performed. In the first case there are subsidies, such as soil fertility, plant density, soil types, soil temperature and deficiency and excess water in the soil can alter the thermal requirements as temperature-base and sum of degree-days (Pascale & Damario, 2010; Renato, Silva, Sedivama, & Pereira, 2013; Schmidt et al., 2017). However, they are general information, without specific examples of species and experimental conditions. In the present case, the magnitude of the differences in the values of temperature-base and sum of degree-days for the different treatments demonstrates to be important to advance in the study of the subject.

Table 2. Sum of degree-days (ºC) of the sub-periods: sowing - emergence; emergency - emission of floral tassel; floral tassel emission - physiological maturation of the species *Cyperus difformis* L. submitted to different soil water conditions: 5.0 cm water depth from sowing = water depth I; water depth applied 21 days after emergence = water depth II; 100% of the water retention capacity (WRC) in the soil = 100% WRC and 50% of the water retention capacity (WRC) in the soil = 50% WRC I. Santa Maria, state Rio Grande do Sul, 2018.

	Experimental time				Average sum of degrees-day (ºC)
	I	II	III	IV	
Water condition	Sowing Sub-period – Emergency Sum of degree-days (ºC)				
Water depth I	39.3	48.3	59.1	48.9	48.9
100% WRC	106.7	93.8	112.1	1131	106.4
Emergency Sub-period - Emission of floral tassel					
Water depth I	610.4	547.6	656.2	664.1	596.8
100% WRC	579.4	591.0	611.5	547.2	582.3
Water depth II	467.6	577.7	597.8	536.4	544.9
50% WRC I	152.8	144.8	229.1	189.4	179.1
Floral Tassel Emission Sub-period - Physiological Maturation					
Water depth I	165.5	180.3	152.6	166.2	166.1
100% WRC	164.5	181.1	190.2	167.6	175.8
Water depth II	238.2	227.3	238.9	225.9	232.6
50% WRC I	230.3	239.4	227.0	195.7	223.1

Experimental times: I = 10/25/16; II = 11/14/16; III = 10/12/16 and IV = 05/01/16.

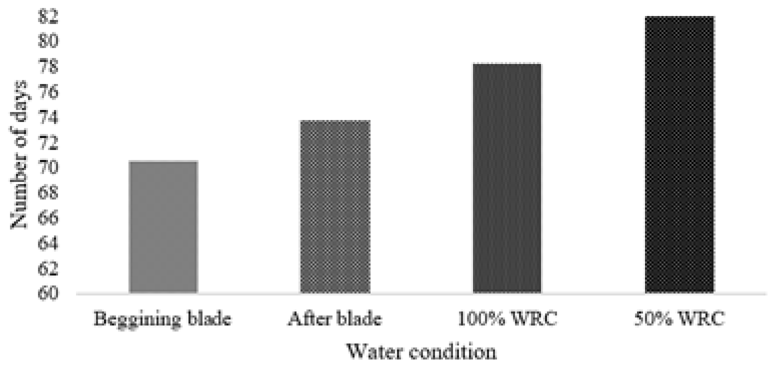

Figure 3. Duration, in days, of the biological cycle of *Cyperus difformis* L. plants submitted to different soil water conditions: soil water depth of 5.0 cm from sowing, 5.0 cm water depth introduced 21 days after emergence 100% water retention capacity (WRC) in the soil and 50% of the water retention capacity (WRC) in soil introduced at 21 DAE. Santa Maria, state Rio Grande do Sul, 2018.

Conclusion

Marshy conditions are more favorable to the growth and development of *Cyperus difformis* plants, accelerating the biological cycle and the intensity of the growth rate.

Different soil water conditions condition the thermal requirements of the sowing-emergence sub-periods, emergence-emission of the floral tassel and emission of the floral-maturing tassel of the *Cyperus difformis* plants, temperature-base varies for each treatment and the sum of degree-days decreases with the water deficit as a function of the high temperature-base, and thus, lower rates of accumulation of sum-thermal.

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