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Water status response of pineapple using destructive and non-destructive indicators and their relations in two contrasting seasons

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ABSTRACT

The use of indicators to assess the water status of pineapple grown inside greenhouses is essential to obtain higher yields. Although destructive and non-destructive indices are widely applied to monitor several crops, there are no previous reports that evaluate their response to different seasons or the relationship between the indicators and the water status of pineapple plants. The experiment was conducted during the warm and cold seasons, comparing two treatments: plants irrigated to field capacity (FC) and non-irrigated (NI). The water status was measured using a destructive index, Relative Water Content (RWC), and non-destructive indices, Normalized Difference Vegetation Index (NDVI), and Stress Degree Day (SDD). The environmental conditions monitored were temperature, humidity, and photosynthetically active radiation (PAR). At the end of each season, plant biomass and assimilate partitioning (AP) were also determined. The recovery of the NI treatment by irrigation was measured using NDVI and RWC. In the cold season, differences between treatments were detected after 45 days of water restriction by NDVI, while in the warm season, the RWC index found differences a week after the experience began ($p < 0.05$). SDD was the most sensitive index since it increased in both seasons and presented significant differences in the early stages of the experiment between treatments in the early stages of the experiment. RWC had intermediate correlations with non-destructive indicators, NDVI and SDD, in both seasons ($p < 0.05$). The AP and its correlation with the water status indices varied between seasons due to different environmental conditions. The RWC and NDVI response to the re-hydration of the substrate in the NI was immediate; however, NDVI values were lower than at the beginning of the experiment. This work highlights the viability of both destructive and non-destructive indicators for the determination of pineapple water status and its relationship with AP in two contrasting seasons.

1. Introduction

The assessment of the water status of vegetation canopies for crop production is of great importance to obtain higher yields. In irrigation management, it is essential to identify the optimal indicators to monitor the water status of the crop. While crop water indicators have been widely investigated for crops grown under open field conditions, the same cannot be said for high value fruit crops grown under greenhouse conditions.

Pineapple [*Ananas comosus* (L.) Merr. var. comosus] is the most economically important species of Bromeliaceae and grown commercially in many tropical and subtropical countries (Botella and

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Smith, 2008). Low temperature is the main limiting factor for the development of this crop in subtropical regions. Growth is delayed between 10 and 16 °C (Carvalho et al., 2005), meaning that the use of greenhouses can eliminate the potential for low temperature stress. However, the adjustment of water supply in this cultivation system is essential, so it is necessary to use parameters to monitor the water status of the crop.

Plants with crassulacean acid metabolism (CAM), such as pineapple, minimize water loss through nocturnal carbon assimilation when temperatures are low, which is reflected in low evapotranspiration (Wai et al., 2017). Pineapple also exhibits substantial C3-type CO_2 uptake in the afternoon when well-watered (Bartholomew and Malézieux, 1994). The mesophyll of pineapple leaves is composed of two clearly differentiated zones: a dark zone formed by chlorophyll parenchyma where the vascular bundles and fibrous caps (sclerenchyma) are located, and a clear area composed of aquiferous parenchyma that is a natural reservoir of water in the leaves (Derwidueé and González, 2010). These features are of great interest when studying water indicators and their interactions.

Several studies have evaluated greenhouse production of pineapple in the subtropical province of Corrientes, Argentina. One of these studies demonstrated that the greenhouse cultivation benefits the vegetative development and photosynthetic activity of pineapple compared to field cultivation (Gómez Herrera et al., 2019). Another study compared two types of water supply, misting and soil irrigation, showing that the most efficient method of applying water and maintaining leaf temperature to avoid heat stress of pineapple plants was soil irrigation (Demarco et al., 2020). Nevertheless, none of these studies identify the changes and relations between different water status indicators during drought stress.

Among the indicators most frequently used to monitor the water status, is the Relative Water Content (RWC) index. The RWC is a useful indicator of the state of water balance of a plant because it expresses the absolute amount of water, which the plant requires to reach artificial full saturation (González and González-Vilar, 2001). Although it is a simple method, it requires a minimum of instruments and time to process the samples, which also damages the leaves.

The Normalized Differential Vegetation Index (NDVI) expresses the ratio of spectral reflectance on the canopy in the infrared and red region and it is used to monitor the effect of water stress on plant growth and forecast biomass. This index is sensitive to different environmental and physiological parameters (Hatfield et al., 2008). The active photosynthetic tissue increases the amount of radiation absorbed in the red spectrum and the energy reflected in the near-infrared (Federer and Tanner, 1966).

Another indicator of water tress is the Stress Degree Day (SDD) introduced by the United States Department of Agriculture (USDA) of Phoenix. It is based on the temperature differences between the canopy and the environment, taking into consideration that plants under conditions of drought stress close the stomata and decrease transpiration with a consequent increase in temperature of the leaves relative to air temperature (Kirkham, 2005).

The benefits of SDD and NDVI are the ease of determination, and being non-destructive methods, allowing to monitor the same individual over time.

The use of several indicators, to monitor irrigation in crops, has been widely investigated; however, there are no previous studies that evaluate their relation to the water status of pineapple plants with CAM metabolism.

The objectives of this study were to analyze the response of water indicators in two contrasting seasons and to determine the relations between destructive and non-destructive indicators of water status in pineapple.

2. Materials and methods

The experiment was conducted at the experimental station of the Universidad Nacional del Nordeste (Corrientes, Argentina; 27°28′ S; 58°49′ W, 70 m a.s.l.).

Pineapple plants of cultivar Smooth Cayenne were grown from 500 to 600 g suckers during six months before beginning the experiment to allow adequate root development. Plants were then transplanted into 3 L plastic pots containing Grow Mix Multipro® medium which is composed by peat moss at 85% to 90% (Terrafertil, Buenos Aires, Argentina).

Plants were grown in a greenhouse, 5.5 m high × 25 m long × 8 m wide, with a 100 μm thick polyethylene covering (Agrotileno, IPESA & Rio Chico S.A., Buenos Aires, Argentina). Temperature and humidity were recorded using datalogger sensor (DAF-10 Data-Logger, Schwyz, China). The values of photosynthetically active radiation (PAR) intercepted by the culture were obtained using a ceptometer (Ceptometer BAR-RAD DUAL, Cavadevices Incorporation, Argentina) with PAR radiation sensors and spectral response in the band between 400 and 700 nm of wavelength. PAR measurements were performed from 8 different points inside the greenhouse and expressed as μmol of photons m^2 $second^{-1}$.

The experiment was divided into two treatments: plants irrigated to field capacity (FC) and non-irrigated (NI). The test was conducted during the cold and warm season. The end of the experiment, for each season, was considered when the pots of NI weight remained constant; the duration of the cold season was 75 days, and in the warm season was 28 days.

The experiment was arranged as a completely randomized design with a sufficient number of plants to allow destructive sampling over time with ten replicates per treatment at each sampling time.

The relative water content (RWC) was determined by removing a 2 cm^2 disc from the center of the "D" leaf, measuring its fresh weight (FW), submerging it in water for 8 h and measuring its turgid weight (TW). The leaf disc was then dried at 70 °C to a constant weight and dry weight (DW) was determined. The RWC was calculated as:

$$RWC(\%) = \frac{FW - DW}{TW - DW}\,x\,100$$

The normalized difference vegetation index (NDVI) expresses the ratio of spectral reflectance on the canopy in the infrared and red region and it is used to monitor the effect of water stress on plant growth and forecast biomass (Romano et al., 2011). The NDVI was measured individually at each plant using a GreenSeeker® Handheld Crop Sensor (Trimble Ag Field Solutions, Sunnyvale, CA, USA) at 8 a.m. and 12 p.m. once a week.

Canopy temperature (Tc) was measured using an infrared thermometer (Tes-1322, Tes Electrical Corporation, Taipei, Taiwan) at 12 p.m. and air temperature (Ta) with a mercury thermometer. Stress degree day (SDD) was calculated as follows:

$$SDD = \sum_{i=0}^{n} Tc - Ta$$

At the end of the experiment thermal digital images of each plant were taken at 12 p.m. with a thermal camera (model C2, Flir Systems, Inc., Wilsonville, OR, USA) as an indication of the temperature from the surface of the foliage.

Plant biomass and assimilate partitioning (AP) were determined by harvesting each plant and individually weighing leaves, stems, and roots to measure fresh weight (FW). Plant organs were then dried in an oven at 70 °C to a constant weight and, leaves, stems, and roots dry weights were determined. The

AP was expressed as g of leaves, stems, or roots per total plant DW (DW plant^{-1}).

Substrate water content (SWC) was measured gravimetrically, weighing every pot individually. It was assumed that the difference in weight among the pots in the NI treatment during the test was due to the water loss (evapotranspiration). The available water (AW) was calculated using the following formulas (Prause, 2006):

$$AW = \frac{FC - PWP}{100} \, x \, AD \, x \, H$$

$$SWC_{initial} = AW$$

$$SWC_n = SWC_{initial} - \frac{((PW_{n-1} - PW_n) \, x \, AD)}{PA}$$

Aw: Available water (mm)
FC: Field capacity (%)
PwP: Permanent wilting point (%)
AD: Apparent density (g cm^{-3})
H: Height of the pot (mm)
SWC_n: Substrate water content at n time (mm)
PA: Pot area (m^2)
PW_n: Pot weight (g), at moment n
PW_{n-1}: Pot weight (g), at moment n-1

At the end of the experiment of the warm season, twenty plants were re-watered, from the water restriction treatment (NI), in order to assess the recovery capacity of the water state of the leaves. RWC and NDVI were measured at 12, 24, 36, and 60 h post-re-watering.

For statistical analysis of the experiment, the traits were assessed using the following linear model:

$$Y_{ij} = \mu + \alpha_i + \lambda_j + \alpha_i\lambda_j + \varepsilon_{ij}$$

where Y_{ij} is the trait to be analyzed, μ is the general mean, α_i is the main effect of the i-th water treatment; λ_j is the main effect of the j-th season; $\alpha_i \lambda_j$ is the interaction effect of the i-th water treatment and the j-th season; ε_{ij} is the experimental error. Prior to comparing the measured variables, normality of the data (Shapiro-Wills test) and homogeneity of variance were tested. Data were compared with ANOVA, Pearson's correlations among traits using InfoStat® software (Di Rienzo, 2019) and means were compared using t-Student test at $p \leq 0.05$.

3. Results

Fig. 1 shows the environmental conditions during the experiment. In the cold season, the relative humidity remained above 80%, and the mean temperature started at 23 °C and then decreased to 15 °C. On the other hand, in the warm season, relative humidity ranged from 66 - 76% and the mean temperature was between 24 and 27 °C. The photosynthetically active radiation (PAR) intercepted by the crop in the warm season varied from 922 to 1377 μmol m^2 s^{-1}, while in the cold season ranged from 601 to 635 μmol m^2 s^{-1} (data not shown).

The means of the indicators measured in this study and significance of main and interaction effects, during both seasons, are presented in Table 1. The water treatment and season significantly affected all four indices. The SDD was lower in field capacity compared to the non-irrigated treatment in both seasons. The NDVI and RWC were higher in the field capacity treatment also in both seasons. The interaction between season and treatment was not significant.

3.1. Relative water content (RWC)

Fig. 2.a shows the RWC results from the cold season. At the beginning of the experiment, both treatments started with 85% of RWC. During the 75 days of the experiment, the FC treatment showed RWC values above the initial one. On the other hand, treatment NI remained stable for 45 days, remaining above 80%. Significant differences were found at 60 and 75 days compared to treatment FC, ending with a difference of 13% at 73% RWC.

The RWC of the "D" leaves in the warm season (Fig. 2.b) in both treatments started with approximately 76%. After seven days of water restriction, significant differences were found between treatments. Along the 28 days of the study, the plants of treatment FC increased their RWC to 85%, while treatment NI it decreased to 65%.

3.2. Normalized difference vegetation index (NDVI)

Fig. 3.a and 3.b present the NDVI values measured at 8 a.m. and 12p.m. respectively during the cold season. At the beginning of the treatments, the initial value of NDVI was 0.72. During the first 30 days of the study, the NDVI values decreased in both treatments and measured times.

After 45 days, significant differences were found between FC and NI treatments at 8 a.m. and 12 p.m. At both measured times,

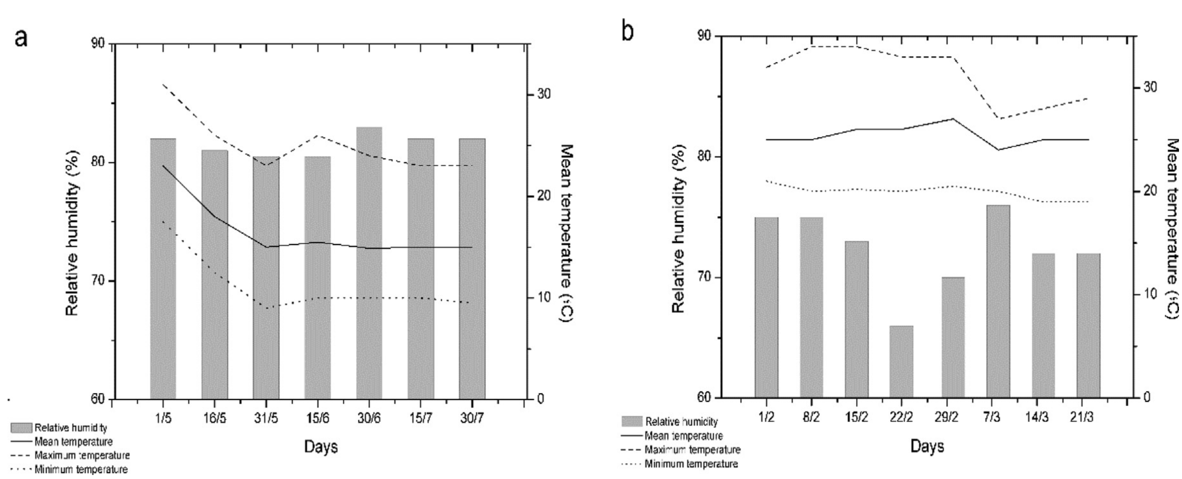

Fig. 1. Mean values of relative humidity, maximum, and minimum average temperatures inside the greenhouse during the cold season (a) and warm season (b).

Table 1
Mean values of the effect of field capacity (FC) and non-irrigated (NI) treatments on Stress Degree Day (SDD), Normalized Difference Vegetation Index (NDVI) measured at 8 a.m. and 12 p.m. and Relative Water Content (RWC).

Seasons	Treatments	SDD (°SDD)	NDVI 8 a.m. (NDVI units)	NDVI 12 p.m. (NDVI units)	RWC (%)
Cold	FC	−5,40	0,67	0,68	86,23
	NI	18,33	0,54	0,58	72,80
Warm	FC	18,10	0,75	0,74	85,22
	NI	41,85	0,64	0,63	64,58
p values					
Treatments		0,0001*	0,0001*	0,0001*	0,0001*
Season		0,0001*	0,0001*	0,0001*	0,0299*
Season × Treatments		ns	ns	ns	ns

ns: not significant.
* Statistically significant ($p \leq 0.05$).

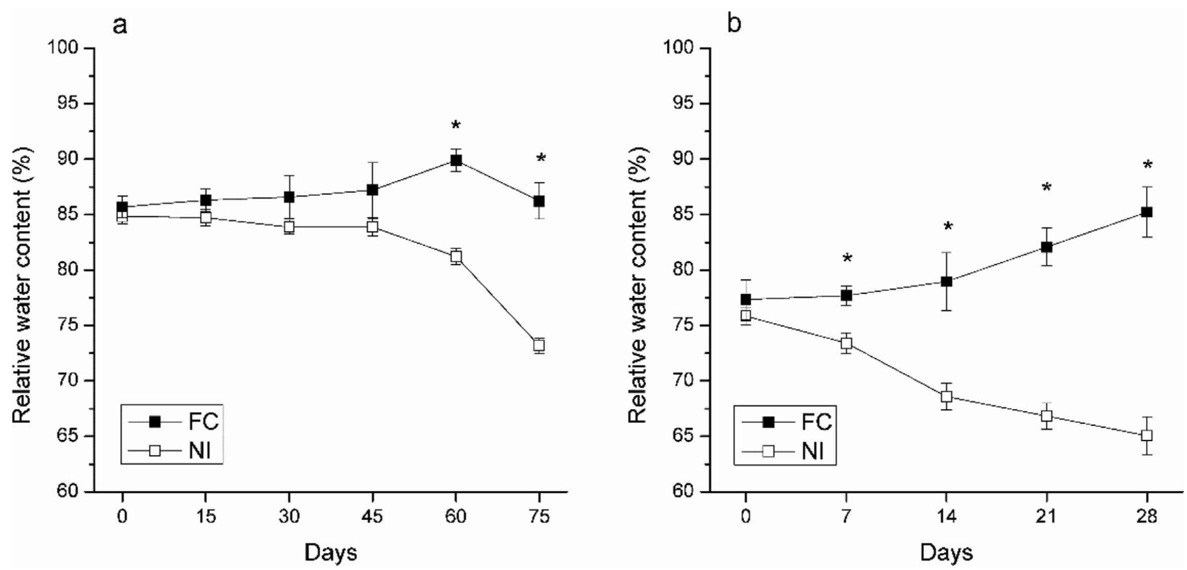

Fig. 2. Relative Water Content (RWC) of pineapple plants exposed to field capacity irrigation (FC) and non-irrigated (NI) during the cold season (a) and warm season (b). Each value represents the mean ± SE of ten replicates. * Statistically significant according to t-Student test ($p \leq 0.05$).

the NDVI values in FC remained above 0.65, while in NI they continued to decrease.

At the end of the experiment (75 days), the NDVI values for FC treatment were 0.67 at both times, while for NI were 0.56 and 0.59 at 8 a.m. and 12 p.m., respectively.

At the beginning of the experiment, NDVI values were above 0.75, in the warm season (Fig. 3.c and 3.d). Significant differences were found between treatments at 14, 21, and 28 days. The NDVI values behavior was similar at 8 a.m. and 12 p.m. reaching 0.63 and 0.62

3.3. Stress degree day (SDD)

Fig. 4.a shows the SDD calculated from the temperature records taken at noon during the cold season. At the beginning of the experiment, the SDD increased from 0 °SDD up to 18.3 °SDD, in conditions of water restriction. The FC treatment presented negative values during the 75 days of this trial, accumulating -5.4 °SDD in the end.

Fig. 4.b shows the SDD of both treatments in the warm season. In the treatment FC, despite being irrigated, the plant showed an accumulation of degrees throughout the 28 days, reaching 18.1 °SDD in that period. On the other hand, in the treatment with water restriction, the accumulation of SDD reached 41.9 °SDD in the same period. The maximum leaf and air temperature differential during this season were 7.9 °C for FC and 16 °C for NI.

Table 2 shows the significant correlations between the water status indicators studied during the cold season. The highest and negative correlation was between SDD and NDVI measured at 8 a.m. Intermediate correlations were found for NDVI and RWC.

The correlations during the warm season are shown in Table 3. There is a high negative correlation between NDVI and SDD. RWC was negatively correlated with SDD and positively correlated with NDVI in both seasons in pineapple plants.

3.4. Thermal images

After 75 days, canopy temperature measured at 12 p.m. during the cold season was 20 °C for the plants in FC and 29 °C for NI treatment (Fig. 5 a.b). While in the warm season, plant temperature was 38 °C in FC and 48 °C in NI treatment (Fig. 5 c.d.).

It can also be appreciated that the substrate inside the pot in the NI treatment had a higher temperature (spots Sp4, Sp5 and Sp6) than the leaves in the warm season (spots Sp1, Sp2 and Sp3).

3.5. Biomass and assimilate partitioning (PA)

Fig. 6.a and 6.b show the biomass and partition of assimilates from the FC and NI treatments obtained in the cold season. The

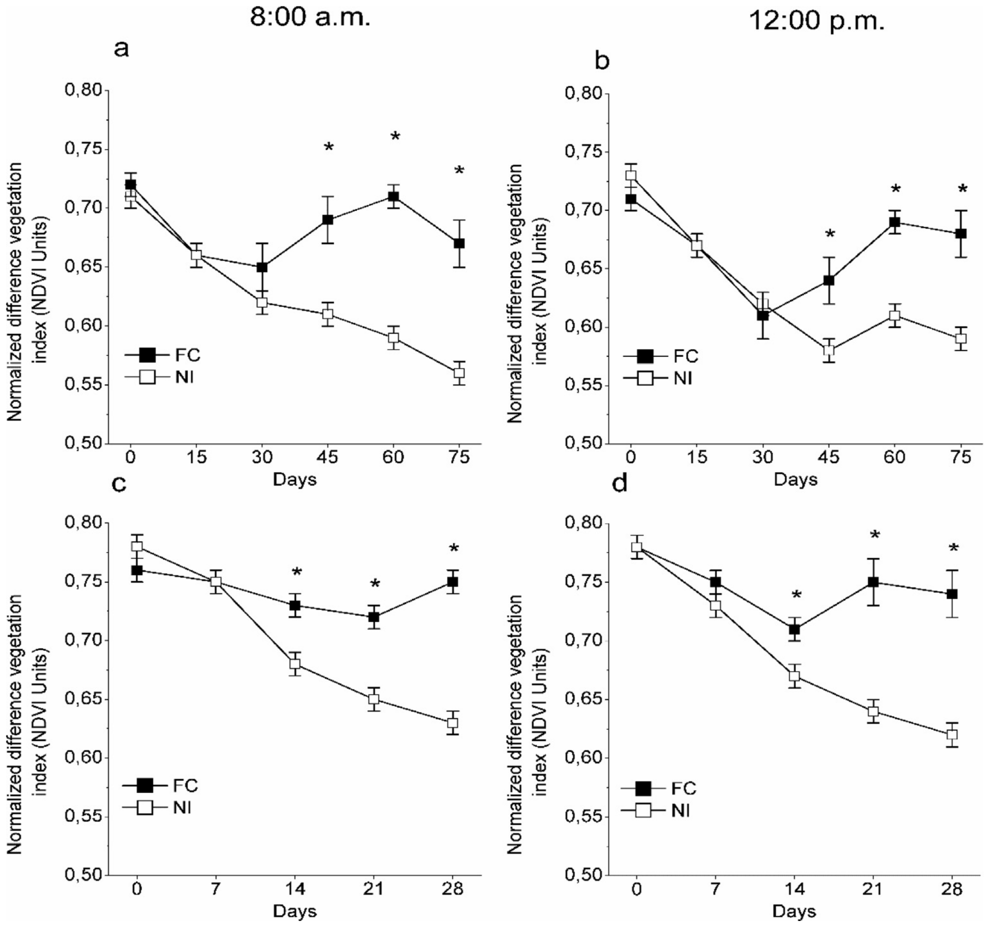

Fig. 3. Normalized Difference Vegetation Index (NDVI) of pineapple plants exposed to field capacity irrigation (FC) and non-irrigated (NI) measured at 8 a.m. and 12 p.m. during the cold season (a, b) and warm season (c, d). Each value represents the mean ± SE of ten replicates. * Statistically significant according to t-Student test ($p \leq 0.05$).

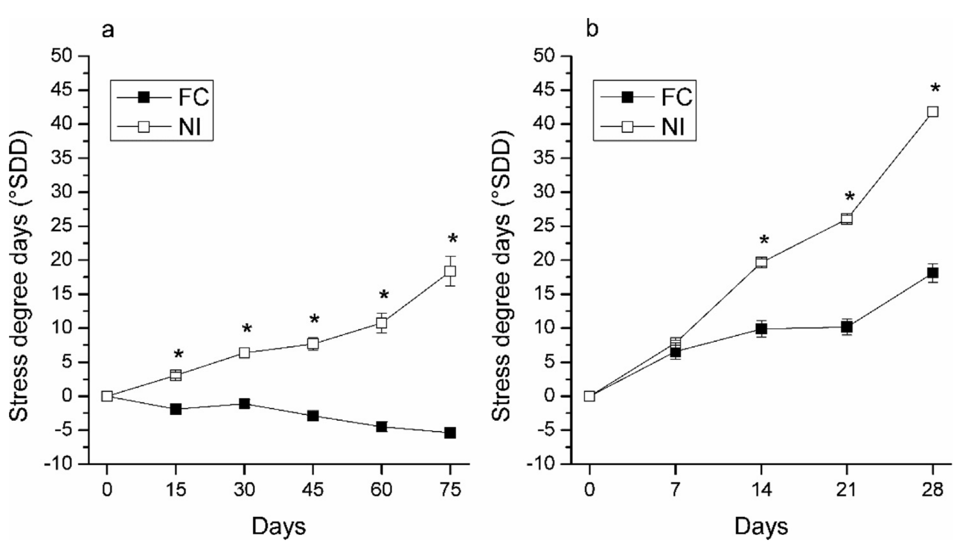

Fig. 4. Stress Degree Day of pineapple plants exposed to field capacity irrigation (FC) and non-irrigated (NI) during the cold season (a) and warm season (b). Each value represents the mean ± SE of ten replicates. * Statistically significant according to t-Student test ($p \leq 0.05$).

treatment FC finished the experiment with 39 g of total biomass while NI reached 41.5 g, without statistical differences. There were no significant differences in the weight of stems and leaves between treatments; however, the roots DW of NI were statistically higher by 5.4% compared to the FC treatment.

In the warm season the final biomass of treatments FC was statistically higher than that of NI (Fig. 6.c). According to the partitioning (Fig. 6.d), the leaves DW in the warm season represented a higher percentage of the total biomass compared to the leaves DW in the cold season (Fig. 6.b)

Table 2
Pearson correlation of water status indicators: Stress Degree Days (SDD), Normalized Difference Vegetation Index (NDVI), Relative Water Content (RWC) during the cold season.

	SDD	NDVI 8 a.m.	NDVI 12 p.m.	RWC
SDD	1			
NDVI 8 a.m.	−0.67*	1		
NDVI 12p.m.	−0.49*	0.78*	1	
RWC	−0.43*	0.47*	0.38*	1

* Statistically significant ($p \leq 0.05$).

Table 3
Pearson correlation of water status indicators: Stress Degree Days (SDD), Normalized Difference Vegetation Index (NDVI), Relative Water Content (RWC) during the warm season.

	SDD	NDVI 8 a.m.	NDVI 12 p.m.	RWC
SDD	1			
NDVI 8 a.m.	−0.73*	1		
NDVI 12p.m.	−0.77*	0.85*	1	
RWC	−0.47*	0.44*	0.50*	1

* Statistically significant ($p \leq 0.05$).

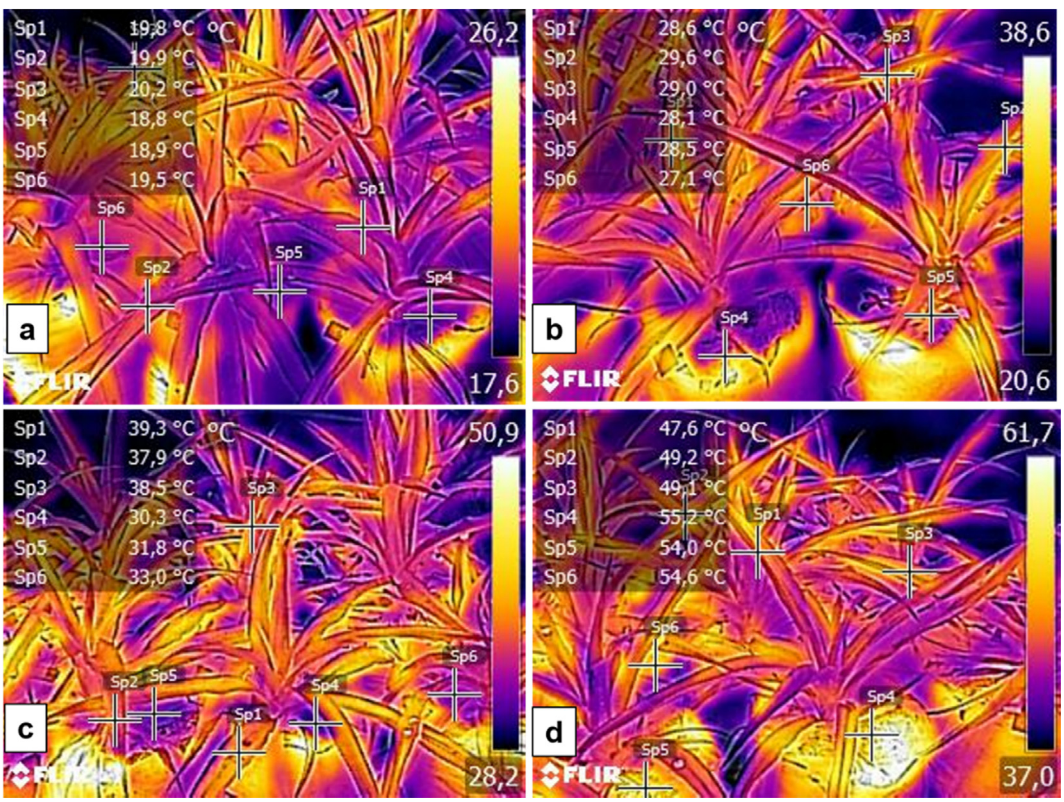

Fig. 5. Thermal images of pineapple plants taken in the field capacity (a) and non-irrigated treatment at the end the cold season (b) and field capacity (c) and non-irrigated treatment at the end of the warm season (d). Leaf and substrate temperatures are shown. Sp1, Sp2 and Sp3 measure the leaf temperature and Sp4, Sp5 and Sp6 measure the temperature inside the pot at three spots in three different plants.

Significant correlations were found, between indicators and biomass, for both seasons (Table 4). During the warm season, a negative correlation was found between SDD and DW of the leaves. Also, there were positive correlations between DW of leaves and NDVI (8 a.m. and 12 p.m.) and RWC. The only correlation of DW of the stem was with RWC, being positive. The DW of the plant had similar correlations than those found in the DW of the leaves. On the other hand, during the cold season, significant correlations were found for other indices. DW of the roots was negatively correlated with NDVI (8 a.m.) and RWC. The only positive correlation of DW of the roots was with SDD.

3.6. Substrate water content (SWC)

Fig. 7. a shows the substrate water content during the cold season, and the decrease of RWC of treatment NI every 15 days, both expressed as a percentage (%) of the initial content. During the first 15 days of the experiment, the water loss from the substrate was 26.6% and 0% of RWC. After 45 days, there was a decrease of 53.7% in SWC and 2% in RWC. At the end of the experiment, there was a total decrease of 67.5% and 13.4%, in SWC and RWC, respectively.

Fig. 7.b shows that both RWC and SWC decreased in the warm season. In the first seven days, the RWC of the leaves decreased by

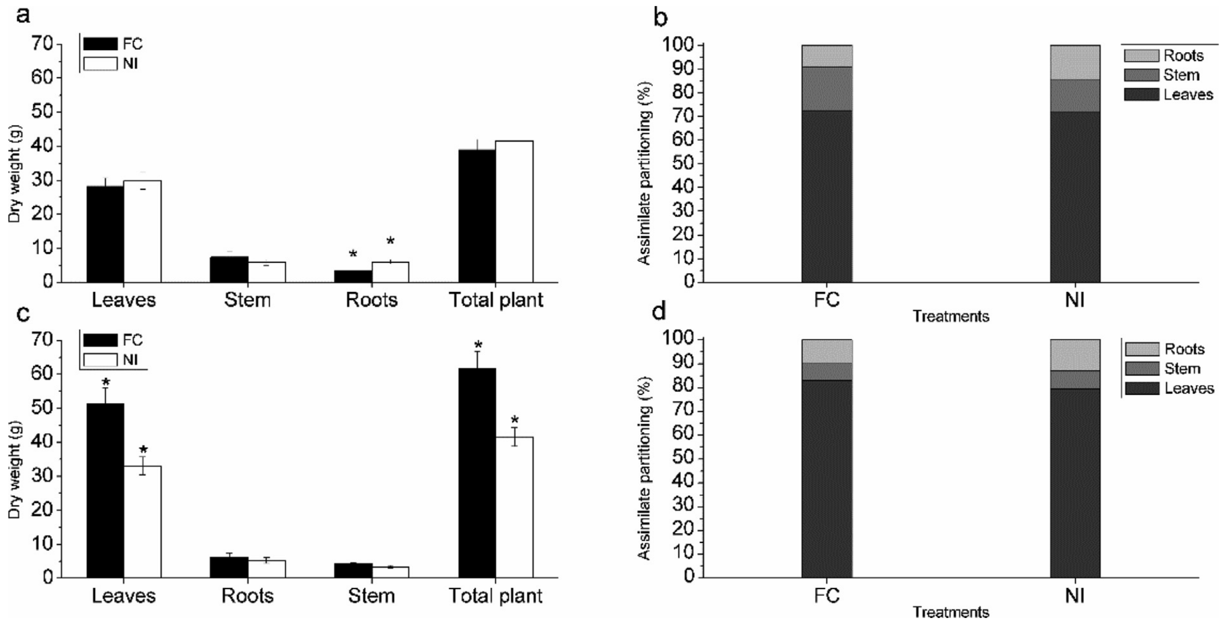

Fig. 6. Final dry weight of leaves, roots, stem, total plant, and assimilate partitioning expressed in percentage for field capacity (FC) and non-irrigated (NI) during the cold season (a, b) and warm season (c, d). Each value represents the mean ± SE of ten replicates. * Statistically significant according to t-Student test ($p \leq 0.05$).

Table 4
Pearson correlation between water indicators and dry weight (DW) of leaves, roots, stem and plant in both seasons.

	Warm season				Cold season			
	SDD	NDVI 8 a.m.	NDVI 12 p.m.	RWC	SDD	NDVI 8 a.m.	NDVI 12 p.m.	RWC
DW leaves	−0.67*	0.39*	0.37*	0.52*	ns	ns	ns	ns
DW roots	ns	ns	ns	ns	0.49*	−0.34*	ns	−0.31*
DW stem	ns	ns	ns	0.39*	ns	ns	ns	ns
DW plant	−0.64*	0.36*	0.39*	0.57*	ns	ns	ns	ns

* Statistically significant ($p \leq 0.05$), ns: non-significant.

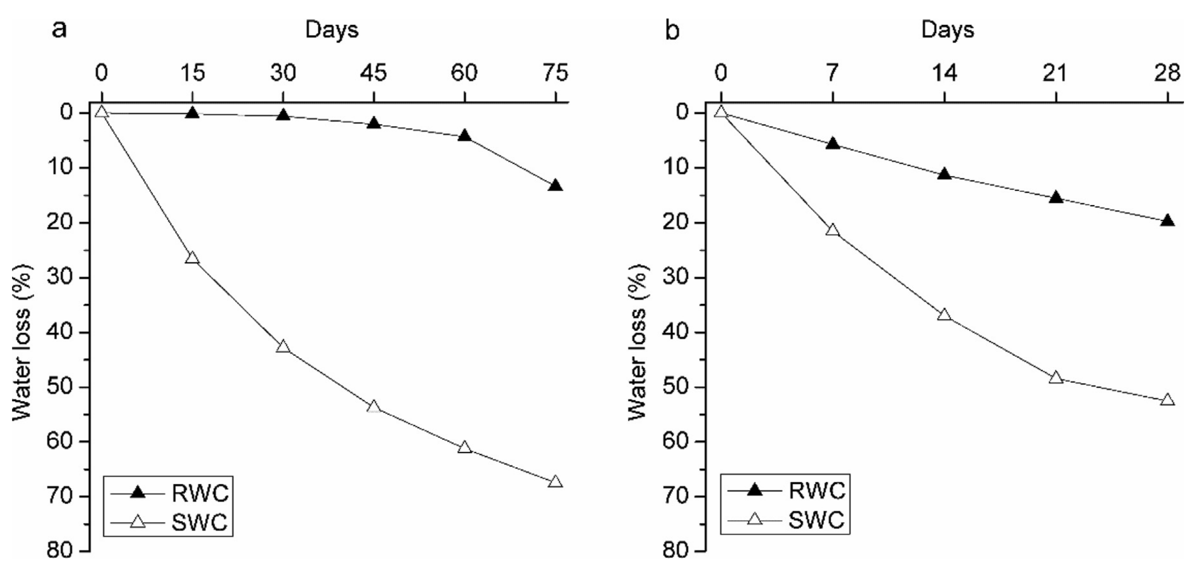

Fig. 7. Decrease in the Relative Content of Water (RWC) and Substrate Water Content (SWC) during the cold season (a) and warm season (b) in treatment NI (non-irrigated), expressed as a percentage of loss.

5%. In the first seven days, the substrate water content decreased by 21.5%, followed by 52.5% after 28 days. At the end of the experiment there was a decreased of 19.8% in RWC.

3.7. Relative water content (RWC) and normalized difference vegetation index (NDVI) response after re-watering the non-irrigated (NI) treatments

Re-watering plants in the NI treatment, the RWC of the leaves recovered from 63.7% to 83.8% (Fig. 8). This water content was maintained, without significant modifications during the following 60 h.

NDVI also responded quickly to the increase in the water content of the substrate, with statistical differences after 12 h (0.68) compared to the initial value (0.62). This index did not change significantly along 60 h, reaching values of 0.70.

4. Discussion

The environmental conditions in the cold season allowed pineapple leaves to retain RWC levels close to 85% for more than 45 days, probably due to decreased photosynthetic activity during low temperatures. The endurance of pineapple leaves to retain RWC levels close to 85% in the non-irrigated treatment for 45 days agrees with the findings of Demarco et al. (2020) for this cultivar. The resistance to dehydration of the plants, shown during the first weeks of drought stress, can be attributed to the presence of the aquiferous parenchyma, one of its xeromorphic anatomical characters that help to preserve the water content in the plant and to minimize water loss through transpiration (Boanares et al., 2018).

If we compare the RWC of the two seasons, in the cold season, differences between treatments were found after 60 days of restriction, while in the warm season, with higher temperatures and faster evaporation of the water content in the pot, the differences were evident just one week after stopping the water supply to the plants. These results demonstrate the strong climate influence on plant water loss, and the importance of irrigation in a rainfed system. Considering the final values of RWC, a 13% difference between treatments was observed after 75 days, while in the warm season a 20% difference occurred within 28 days.

According to Aguilar et al. (2012), NDVI is based on differences in reflectance in the red region (due to pigment absorption) and maximum reflectance in the near infrared (caused by cellular structure). NDVI determinations are currently a widely accepted system for non-destructively monitoring plants and for estimating stress conditions in different species (Balasundram et al., 2013; Mazzetto et al., 2009; Neiff et al., 2015). Ihuoma and Madramootoo (2017) stated that NDVI is also an indicator of the chlorophyll concentration, which has been reported to reduce in pineapple plants during the cold seasons (Ebel et al., 2016; Rebolledo Martínez et al., 2002). So, the decrease of NDVI, observed in this study during the first 30 days with low temperatures, was expected. Demarco et al. (2020) also reported significant differences for NDVI between different types of water supply in pineapple after two weeks treatments. Our results suggest that the sensitivity of NDVI is higher in the warm season than in the cold one, when pineapple plants present lower photosynthetic activity in response to lower temperatures.

The treatment irrigated to FC did not accumulate degrees, but on the contrary, it showed negative values of SDD in the cold season. Pineapple plants have a CAM metabolism, with closing of stomata during the day. However, under specific environmental conditions, pineapple plants may exhibit substantial C3-type CO_2 uptake in the afternoon when well-watered (Bartholomew and Malézieux, 1994). In this study, as the temperature was measured at noon, leaf cooling through the opening of the stomata is less probable. However, it is possible that the negative values of SDD in the cold season had its origin in the higher thermal diffusivity of the potting substrate of the well-watered plants compared to the non-irrigated treatment. Irrigated plants needed higher temperatures to increase the thermal diffusivity of the substrate to conduct the heat to the plants and increase the canopy temperature (Al Nakshabandi and Kohnke, 1965). This study demonstrates

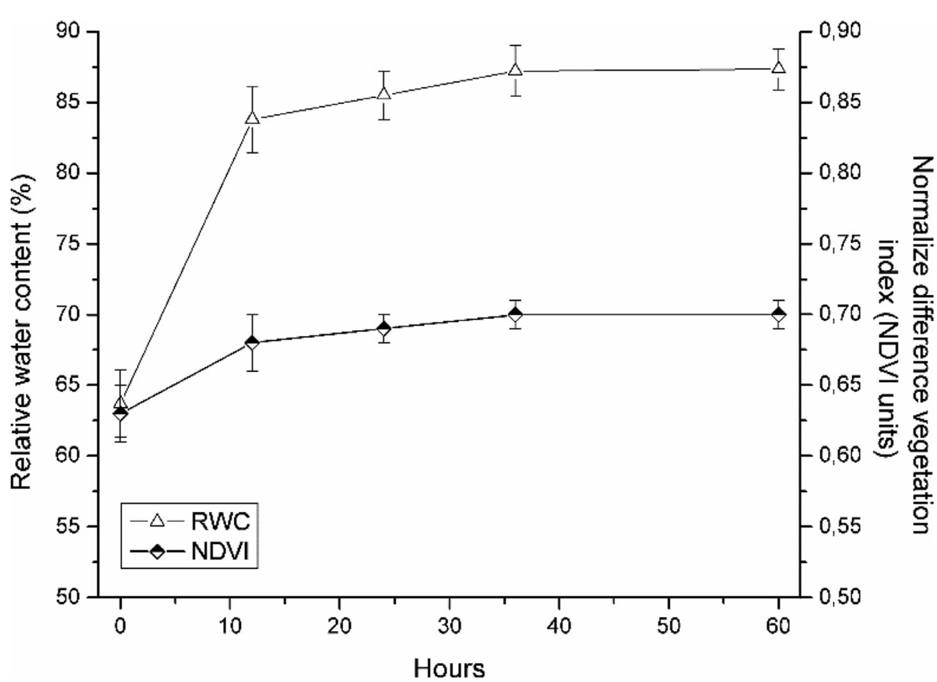

Fig. 8. The Relative Water Content (RWC) and Normalized Differential Vegetation Index (NDVI) response to the re-hydration of the substrate in the warm season. Each value represents the mean ± SE of twenty replicates.

that by maintaining the system at field capacity, the plant does not raise its temperature during the cold season.

The accumulation of SDD in the warm season is probably due not only to the restriction of water supply but also to the high temperatures, with the maxima of this period oscillating between 28 and 35 °C. Zhu et al. (2002) studying the photosynthetic gas exchange for pineapple grown under ambient and elevated CO_2 and three day/night temperature, observed leaf temperatures 7 to 9 °C above air temperature during the day in all temperature treatments.

Previous studies with cotton (*Gossypium hirsutum* L.) applying three irrigation treatments (well-watered, moderately stress and severe stress) showed similar results to those found in this study during the cold season. The well-watered plots had canopy temperature below ambient temperature, while the stressed crop showed mostly positive differences indicating elevated canopy temperatures with respect to ambient temperature (Bajwa and Vories, 2007). Likewise, a study carried out on wheat (*Triticum aestivum* L. var. RR-21), had three irrigation levels: well-watered, half irrigated, and without irrigation. The results showed that the SDD values decreased rapidly in the presence of irrigation (Kumar and Tripathi, 1990). For pineapple, there is no other record of the use of SDD as an indicator of drought stress available for comparisons with our results.

Canopy temperature by thermal imaging is a useful tool to study plant water status and estimate other crop traits (Romero-Bravo et al., 2019). In the cold season, the negative SDD values showed that plants were cooler than the air temperature in the FC treatment. If we compare the thermal images of this treatment with the general average temperature of the cold season, there is higher canopy temperature. However, the average maximum temperature inside the greenhouse in this season is reached from 12 p.m. to 2 p.m., which is about the time when the SDD and thermal images are taken. At that time period, the plant canopy temperature obtained by thermal images is lower than the average maximum temperature, giving a negative value for this difference.

At the end of the warm season, both treatments studied showed positive SDD. By closing the stomata, the canopy temperature increased, to a larger extent in the NI treatment. The canopy temperatures obtained by thermal images in this study agree with the findings of the Demarco et al. (2020) who stated that that water supply by soil application in pineapple, grown under protected environmental conditions in subtropical regions, maintains plant growth and prevents excessively high leaf temperatures during hot seasons.

The difference in root growth observed between treatments during the cold season may be due to different acclimatization mechanisms that are activated by water stress (Basu et al., 2016). The availability of water affects the relationship between shoot and root growth; the root continues its growth searching water while the shoot stops growing due to stress (Potters et al., 2007; Shao et al., 2008). The increased leaves biomass of FC, during the warm season, can be attributed an adequate water supply, optimum temperatures for plant growth and higher photosynthetically active radiation during this period (Manrique et al., 1991). In the warm season, biomass increase was dependent on the DW of leaves, while at the cold season on the DW of roots.

Although in the FC treatment in both seasons the plants were not subjected to drought stress, their DW was higher in the warm season than in the cold one. Temperatures below 16 °C tend to stop pineapple growth (Carvalho et al., 2005). Plant final size for the FC treatment was 61.7 g in the warm season and 39 g in the cold season. The environmental variables that influenced dry weight were higher temperature and photosynthetically active radiation as, in both seasons, pineapple plants had an adequate water supply to maintain the substrate at field capacity. On the contrary, plants of the NI treatment in both seasons weighted 41.5 g. Although the warm season had temperatures adequate for growth, the lack of water was growth limiting. These results show that the main limitation in the NI treatment was water availability restriction.

The correlations between water status indicators in this study varied according to the seasons. RWC was best correlated with NDVI measured at 8 a.m ($r = 0.47$, $p < 0.05$) in the cold season, while in the warm season was with NDVI 12 p.m. ($r = 0.50$, $p < 0.05$). During the cold season a negative correlation was found between SDD and NDVI 8 a.m ($r = -0.67$, $p < 0.05$), whereas in the warm season was with NDVI measured at 12 p.m. ($r = -0.77$, $p < 0.05$). The difference between seasons and NDVI values at 8 a.m. to 12 p.m, can be attributed to changes in chlorophyll and nitrogen content of the tissues (Sideris et al., 1948; Ebel et al., 2016). The RWC was negatively correlated with SDD, showing similar values in both seasons, -0.43 ($p < 0.05$) in the cold season and -0.47 ($p < 0.05$) in the warm season. This relation was expected, as temperature increases determine rising evapotranspiration and hence tissue water content decreases (Ihuoma and Madramootoo, 2017).

Both seasons also influenced the correlation of the assimilate partitioning and water status indicators. In the cold season, negative correlations were found between DW of roots with NDVI 8 a.m. ($r = -0.34$, $p < 0.05$), and RWC ($r = -0.31$, $p < 0.05$). During this period, low temperatures caused plants to stop growing; however, DW of roots was statistically higher in the non-irrigated treatment. This result would explain the reason for the negative correlation between the DW of roots with RWC. There was also an intermediate correlation between the DW of roots and SDD ($r = 0.49$, $p < 0.05$). This positive correlation can be attributed to the increasing SDD values and DW of roots in the NI treatment. On the other hand, the warm season showed different behavior in the correlation of the assimilate partitioning and water status indicators. The correlations were very similar when comparing DW of plant and leaves with SDD, NDVI, and RWC. The SDD was negatively correlated with DW of leaves ($r = -0.67$, $p < 0.05$) and plant ($r = -0.64$, $p < 0.05$). Plant growth was negatively affected by higher temperatures in this season and increased canopy temperature in the NI treatment. While RWC had negative correlations in the cold season, in the warm season showed intermediate positive correlations with DW of leaves ($r = 0.52$, $p < 0.05$), plant ($r = 0.57$, $p < 0.05$) and stem ($r = 0.39$, $p < 0.05$). These relations, differently from the cold season, can be attributed to the higher DW of plants and leaves in the FC treatments determined by environmental factors in the warm season.

The RWC response to the re-watering of the substrate was immediate (Fig. 8), returning to values similar to those shown in its initial state. Bonet Pérez et al. (2010) reported that the effect of drought in this species is reversible, and when the water is available again, the leaves are rehydrated and continue to grow. NDVI also responded quickly to re-watering, but values were lower than those of its initial state, possibly due to chlorophyll damage caused by the severe drought stress.

5. Conclusions

Both the non-destructive (NDVI – Normalized Difference Vegetation Index; SDD – Stress Degree Days) and destructive (RWC – Relative Water Content) indicators studied showed to be viable for the determination of pineapple water status. However, these indices responded differently according to the seasons and water treatments studied.

The SDD was the most sensitive indicator of pineapple water status, increasing in the cold and the warm seasons at early stages of drought stress. It presented a high negative correlation with NDVI during the warm season.

NDVI was more sensitive in the cold season and RWC in the warm season, and both responded quickly to re-watering the plants that were subjected to water stress.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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