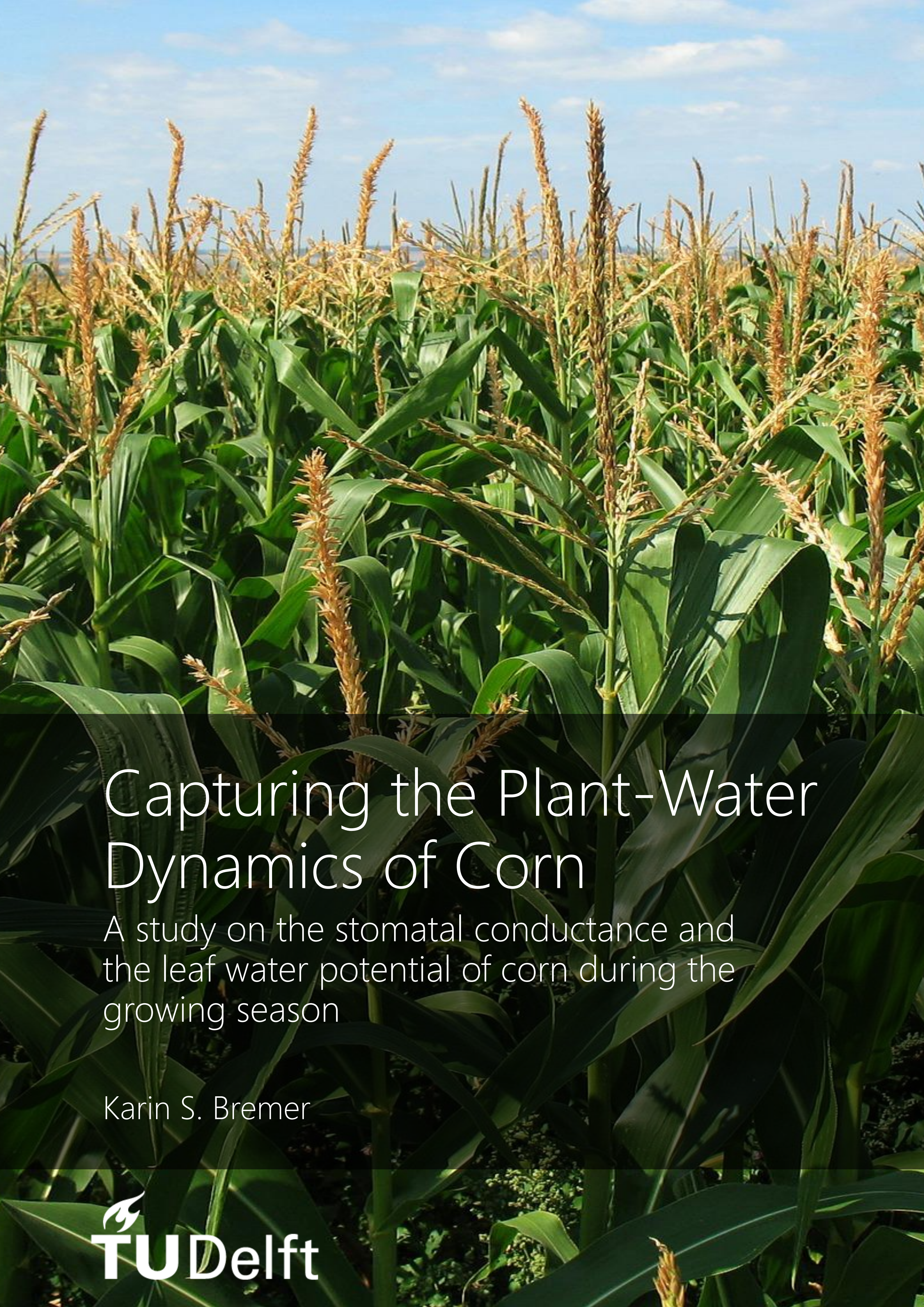

Capturing the Plant-Water Dynamics of Corn

A study on the stomatal conductance and the leaf water potential of corn during the growing season

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by

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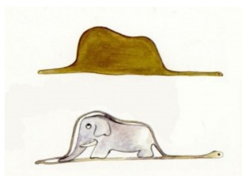
K.S. Bremer
Delft, February 2019



When eating an elephant
take one bite at a time.
-
Or not...

Summary

Remote sensing is used for monitoring crop growth. However, most currently used remote sensing methods are limited by cloud cover. Radar remote sensing has as advantage that it is not limited by cloud cover. The use of radar for crop monitoring is currently being researched. With radar, moisture content can be observed, including the moisture content in the vegetation. This could make radar a useful tool to detect water stress in vegetation. Radar can penetrate through the vegetation at different heights and can therefore observe variations in water content over height. To be able to detect water stress with radar, knowledge on the water distribution in for well-watered and water stressed vegetation is needed. The water distribution is a result of the plant-water dynamics of a plant and the factors influencing these dynamics. There are two characteristics that give important information on the plant-water dynamics: the stomatal conductance and the leaf water potential. The stomatal conductance is a measure of the water vapour exiting though the small pores on the leaf. The water transport through a plant is driven by the difference in water potential between the soil and the atmosphere. The leaf water potential is a measure of the water stress a plant experience. This study aims to characterize the variation in stomatal conductance and leaf water potential of corn plant in height over time on a diurnal time-scale and on a seasonal time-scale, under well-watered and water stressed conditions. An additional objective was the development of a protocol for plant-water relation measurements in radar experiments.

Field experiments were done to measure the leaf water potential by conducting pre-dawn measurements three times a week, evening measurements once a week and a mid-day measurement in the beginning and at the end of the growing season. The stomatal conductance was measured multiple times per day for three days a week, given that there was no precipitation. As the research is part of a larger project, additional hydrological data, soil moisture data and sap flow data were collected.

Due to unforeseen circumstances and an exceptional wet season, it was not possible to impose water stress. The measurements were therefore only done under well-watered conditions, no information on the plant-water relations under water-stressed conditions could be obtained. For the stomatal conductance a clear variation over height was observed. This variation was caused by limited solar radiation for the lower leaves. The leaves that received full solar radiation had clear diurnal cycle in stomatal conductance and a high variation in stomatal conductance. In water-stressed conditions, it is expected to see a change in stomatal behaviour in these leaves. It is therefore recommended to focus on these leaves under water-stressed conditions. For the leaf water potential, no values were reached that have been connected to water stress in the literature. Also, no water stress coping mechanisms were observed in the corn. From this it can be concluded that no water stress took place during this experiment for the days on which data was collected. In the leaf water potential data a clear influence of the soil water potential was observed. When the soil water potential was high, close to zero, the pre-dawn leaf water potential was also high. For days with low soil water potential, a decrease in leaf water potential was observed. During the day, a clear diurnal cycle was observed. On days with high evaporation, the leaf water potential values observed during the day were lower compared to days with lower evaporation. In general, the variation over height was small on the observed days. A slight decrease in leaf water potential over height was observed. The difference over height increased more as the leaf water potential was lower.

This study gives insight in the stomatal conductance and leaf water potential in well-watered field conditions, and how these are influenced by the environmental factors. However, for the purpose of capturing the plant-water dynamics for radar experiments it is recommended to conduct a similar study for water-stressed corn.

Contents

List of Figures

List of Tables

1 Introduction

Irrigation in agriculture is the main user of fresh water resources, since it accounts for around 70% of the total withdrawal (Programme)/UN-Water, 2018). The growth of vegetation depends on several inputs, like nutrients, sunlight and water. A shortage or a surplus of one of these inputs limits the growth of the plant. Water stress is one of the main limiting factors for yield production. Therefore it is desired from a food production perspective to limit the water stress of crops. However, from a water management perspective efficient use of water is desired to prevent unwanted water losses. Remote sensing is a useful tool for crop monitoring. Various ways are used to detect water stress in plants with the use of remote sensing, such as the leaf/canopy temperature, near infra red (NIR), normalized difference vegetation index (NDVI) and other spectral indices, fluorescence and radar (Cozzolino, 2017; Ma et al., 2018; Petruzzellis et al., 2018; Steele-Dunne et al., 2016; Zarco-tejada et al., 2019). The use of radar to detect water stress is currently researched further (Steele-Dunne et al., 2012; 2016).

One advantage of the use of radar is that the microwaves can penetrate clouds because of its low-frequency wavelengths ($\lambda = 0.1 - 100 cm$). Also no illumination from the sun is needed, making it possible to have measurements during day and night. The radar backscatter of vegetation is determined by the system parameters, e.g. the frequency, polarization, incidence angle and azimuth angle, and on the surface characteristics, e.g. soil roughness, vegetation geometry, the dielectric properties of the soil and, for this study most importantly, the dielectric properties of the vegetation. The dielectric properties of vegetation depend on water content, temperature and salinity, of which the water content has the most influence. This makes radar a useful tool to detect canopy moisture content.

The established methods for the detection of the moistaure content in vegetation by radar were developed around 40 years ago. One of these methods is the water cloud model (WCM), which models the moisture in the vegetation as a uniformly distributed cloud that grows during the growing season of the crop (Ulaby et al., 1986)(as cited in (Steele-Dunne et al., 2016)). However, as Steele-Dunne et al. (2016) found, in reality the moisture content in the canopy is not uniformly distributed. In fact, for well-watered corn the moisture content in the stem decreases over height, while the leaves in the middle section of the plant are the leaves with the highest water content. The non-uniformity in fully grown corn was also observed by Joerg et al. (2018) in the backscatter of radar measurements. For observations with a short wavelength a high backscatter was observed in the corn canopy at a height between 1 and 1.5 m, while the observations with a longer wavelength showed little variation in backscatter over height.

A previous study by Van Emmerik et al. (2017) has shown that the dielectric properties of maize leaves differ for leaves on different heights and that there is a difference in the dynamics between stressed and non-stressed corn. Especially in water stressed crops a difference in dielectric properties was found between the leaves at different heights and between the 6 A.M. and 6 P.M. The study shows a dynamic response of the dielectric properties on water stress, that is variable in time and space. The clear effect of water stress on the dielectric response of corn leaves at different heights of the canopy as described by Van Emmerik et al. (2017). Therefore, not only the water distribution in a plant can be detected with radar, but also the detection of water stress in a plant might be possible.

The larger project where this research is part of has the goal to further develop radar as a method to detect water stress. When the change of the water distribution in a water stressed crop is known, the radar configurations can be optimized for water stress detection. For example, a longer wave length can penetrate deeper and is therefore able to detect the dielectric properties of the vegetation closer to the ground. In order to understand the dynamic response of the dielectric properties to water stress through the growing season, more knowledge on: (A) water content distribution, (B) plant-water dynamics in stressed and unstressed plants, and (C) the factors controlling and influencing the water transport through a plant is necessary. This research focusses on characterizing (B) and (C), in such a way that it helps to understand the observations done by radar.

This is done by focussing on two measurable variables regarding the water dynamics in a plant: the stomatal conductance and the leaf water potential. These two variables play are of importance for the plant-water dynamics and are regulated by complex processes. The stomata are small pores on the leaf surface. They play an important role for the photosynthesis by regulating the CO_2 influx. However, when the stomata open to let CO_2 in, H_2O leaves the cell, driven by a water potential difference between the leaf and the atmosphere. Because of this, the stomata are the major gateway for transpiration water loss (Lambers et al., 2008). For photosynthesis, irradiation is needed , which in the field is provided by solar radiation (Araújo et al., 2011; Assmann and Shimazaki, 1999; Lambers et al., 2008). The solar radiation cycle causes a diurnal cycle in transpiration. Since there is no solar radiation at night, the stomata are closed and therefore, the transpiration is low. Also, if a plant experiences water stress, the stomata will close to minimize the water loss (Dwyer and Stewart, 1984; Turner, 1974; Yan et al., 2017). The water transport through the plant is driven by a difference in water potential (ψ_w) between the soil and the atmosphere. Water moves from a high potential to a low potential. In a plant, the water moves from the soil, through the roots, up the stem and via the leaves into the atmosphere (Damm et al., 2018; Lambers et al., 2008; Nobel, 2009; Ray, 1972). In the leaf water potential, a diurnal cycle can also be observed which is driven by the diurnal cycle of transpiration. Because the difference in water potential drives the transport of water through the plant, the leaf water potential is an important indicator when analysing water-stress in a plant (Lambers et al., 2008; Ray, 1972). A low leaf water potential is one of the drivers for early stomatal closure (Boyer, 1970; Turner, 1974).

Van Emmerik et al. (2015) concluded that more data on the diurnal variation in the vegetative water content (VWC) over the growing season is needed to fully understand the influence of the VWC on the radar backscatter. As described above, the stomatal conductance and the leaf water potential are two variables that influence the water transport through the plant and that show if a plant has water-stress. Measuring these over the growing season during the day, at pre-dawn and mid-day, and especially at different heights provide the needed information.

Both the stomatal conductance and the leaf water potential of corn have been measured in previous studies under both well-watered and water-stressed conditions (Reicosky and Lambert, 1978; Riboldi et al., 2016; Turner, 1973; 1974; Vanaja et al., 2011). The relation between stomatal behavior and leaf water potential has also been discussed.In most studies, measurements were taken of leaves at same height. Although some studies were performed at different heights of the plant, these took place on fully grown plants (Turner, 1973; 1974). However, in the studies that took place over the growing season, the height of the plant was not taken into account (Dwyer and Stewart, 1984; Riboldi et al., 2016). So far, no study has been found where the seasonal changes in stomatal behavior and leaf water potential have been measured at different heights of the corn plants.

The current studies do not have the aim to characterize the difference between water-stressed and non-water-stressed crops in the stomatal conductance and the leaf water potential at different heights. From a hydrology perspective, it is interesting to get more insight into the dynamics of water transport through plants. This can be done by identifying the variation in water potential over the height of a plant, and the variation in the water flux through the stomata over the height of the plant in different growth stages. For radar experiments, knowledge on the variation in the vegetative water content over the height of the corn plant, both diurnal and at different growth stages, is of importance. The water dynamics of vegetation in relation to radar measurements has not been measured before.

1.1. Research objective and questions

The aim of this research is to characterize the variation in stomatal conductance and leaf water potential of corn plants in height and time through the growing season and under well-watered and water stressed conditions. A key related aim is to develop a protocol for capturing the stomatal conductance and leaf water potential in plants in radar experiments.

The aim can be reached through answering a set of sub questions. To answer these questions the diurnal cyclus of the leaf water potential and of the stomatal conductance will be measured in the field. In order to write a protocol, we need to know how to decide within the limited amount of time in the field what should be measured. The first three sub questions play an important role in deciding this.

1. What is the variation of the stomatal conductance and leaf water potential over the height of the plant?
2. What is the diurnal cycle of the stomatal conductance and the leaf water potential of corn, in well-watered and water-stressed plants?
3. How do the diurnal cycles in stomatal conductance and leaf water potential change over the growing season?
4. How do the changes in the stomatal conductance relate to changes in the leaf water potential and the other way around?

1.2. Thesis outline

More background information on the stomata and leaf water potential is given in Chapter 2. In Chapter 3 the methods and materials used for this research are described. This includes the materials used and the used protocol for the stomatal conductance measurements and the leaf water potential measurements. The results are analysed and discussed in Chapter 4. In Chapter 5 recommendations are given for the design and protocol of future field experiments on stomatal conductance and leaf water potential that are related to radar experiments. Finally, in Chapter 6 the conclusions of this research are summarized and recommendations for future research are given.

2 Theoretical background

The water dynamics in a plant are influenced by many factors. To get a better understanding of what is happening, which factors are important to measure or consider while conducting field measurements and to determine when water stress might play a role, a certain background knowledge is desired. This chapter provides a brief background information on the leaf water potential, the stomata and different factors controlling these.

2.1. Leaf water potential

The water potential in a plant is build up from the hydrostatic potential, ψ_P, the osmotic potential ψ_Π, and the gravitational potential, ψ_h, see Equation 2.1:

$$\psi = \psi_P + \psi_\Pi + \rho_w g h \tag{2.1}$$

The physical pressure that the system applies on the water can be positive or negative. The osmotic potential is determined by the presence of solutes in water. Pure water has a ψ_Π of zero, the more solutes, the lower ψ_Π. This potential allows liquids to flow into opposite direction of ψ_P. The gravitational potential is determined by the density of water, ρ_w, the gravitational constant, g and the height in meters. The decrease in the leaf water potential over height is 0.0098 MPa/m ($\rho_w g$) (Lambers et al., 2008; Nobel, 2009).

A plant can be compared to a pump, moving water from the soil into the atmosphere. The water transport is driven by the potential difference within a plant. This is determined by difference in soil water potential, ψ_s, and water potential in the atmosphere, ψ_{atm}, see Equation 2.2 .

$$\psi_s > \psi_{roots} > \psi_{stem} > \psi_l > \psi_{atm} \tag{2.2}$$

Figure 2.1 shows a schematic drawing of the water flow from the soil, through the plant in the atmosphere. The values mentioned are an indication, given by Nobel (2009), and may vary between species and under different environmental conditions. The highest gradient difference in water potential is the one between the leaves and the atmosphere. The movement of water from the leaves into the atmosphere goes through open stomata.

The ψ_l of a plant is controlled by two external factors: the soil water potential and the transpiration. As mentioned in the introduction, water is sucked up in the plant due to a difference in water potential between the soil and the atmosphere. The soil water potential, therefore, influences the plant water potential, and hence ψ_l. During night, stomata close as there is no solar radiation. Therefore, ψ_l increases over night. At pre-dawn, ψ_l is closest to equilibrium with the soil water potential, making this an important time for measurements (Lambers et al., 2008).

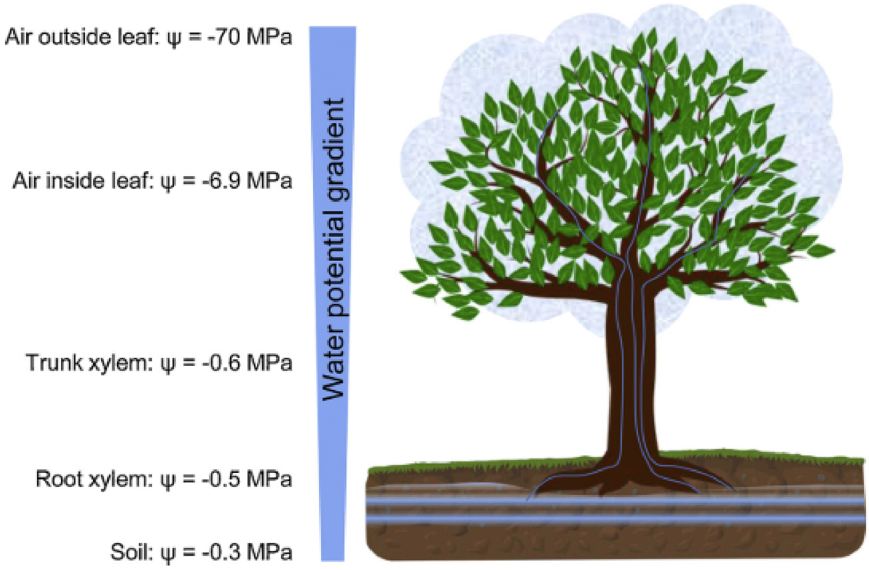


Figure 2.1: Schematic drawing of water potential gradient between soil, plant and atmosphere. The water potential values are representable for several species (Nobel, 2009). Figure originally from Damm et al. (2018).

Where soil water is the source of water in the plant, transpiration is the cause of loss of plant water. The transpiration causes a decrease in the plant water potential and is therefore the major driving force of water through the plant. The transpiration is driven by solar radiation. This causes a diurnal cycle in the transpiration, and therefore also a diurnal cycle in the leaf water potential. The lowest ψ_l occurs around mid-day when the transpiration is highest (Lambers et al., 2008; Turner, 1974).

Besides the soil water potential and the transpiration, ψ_l is also influenced by the height of the leaf, as is shown in Equation 2.1. The effect of height on ψ_l in corn plants is very small. With a highest measured leaf at 1.5 m, the influence of leaf height on the leaf water potential is assumed to be small and will therefore be neglected. Last, the measurement location on the leaf also influences the leaf water potential. In most research, the in-leaf water potential gradient is not taken into account, or is assumed to be equal over the leaf length (Neumann et al., 1974; Turner, 1973; 1974). Research on the in-leaf water potential gradients was conducted by Wiebe and Prosser (1977). They found that the ψ_l of a corn leaf is lower ψ_l in the tip and higher near the base. The ψ_l at the mid-tip and mid-base were similar in well-watered conditions. When the plant experienced water stress, ψ_l in the tip decreased, and ψ_l in the mid-tip became more equal to that in the tip. After increasing the water availability again, ψ_l at the tip did not recover, while the other parts of the leaf did. The cells in the tip started to die because of the limited water availability.

2.2. Stomatal behaviour

Transpiration occurs through open stomata in combination with vapour pressure deficit (VPD). The stoma (plural "stomata") consist of two guard cells above a cavity. When the guard cells swell, the stomata open. The stomata close when the guard cells loose water and shrink. The distribution of the stomata on a leaf vary. For corn leaves, more stomata are found on the bottom (abaxial) leaf surface, then on the top (adaxial) leaf surface (Nobel, 2009), Driscoll et al. (2006) and (Slavik (1963), as cited in Jarvis (1986)).

Stomatal conductance is a measure of the rate of water vapour exciting the stomata in $mmol/m^2s$. It is influenced by various factors, such as solar irradiance, the CO_2 concentration, the air quality and humidity, water stress and endogenous plant hormones. However, there is still much unknown on the exact mechanism with which the environmental factors regulate to the opening and closing of the stomata and the diurnal variation in this (Araújo et al., 2011; Lambers et al., 2008). The most important factors influencing the stomatal conductance are discussed below, including how these play a role in this research.

Radiation is the major factor controlling stomatal behaviour. In the case of field experiments, this radiation is solar radiation. However, other blue or red light will have the same effects (Araújo et al., 2011; Shimazaki et al., 2007) During seasonal *in vivo* stomatal conductance measurements, the solar radiation on the measured leaves is variable and determined and influenced by various factors, such as the time of the day, cloud cover and seasonal changes in solar radiation, but also by the leaf cover and leaf orientation of the measured leaf.

The diurnal pattern in solar radiation causes the diurnal cycle in stomatal aperture. This diurnal cycle can be influenced by cloud cover. Depending on the thickness of the cloud, it limits the solar radiation on leaves in the field. The diurnal cycle of solar radiation also changes throughout the year, as the days are long in the summer and short in winter. This change increases for locations further from the equator.

Besides these environmental factors, the vegetation itself also influences the radiation received by the leaves. The shading caused by leaf cover is an important factor in the variation of stomatal conductance over height. As the plants grow, the leaf area of the crop increases, and the solar radiation is blocked form the lower leaves by leaves higher in the canopy. Some factors determining the leaf cover are: The leaf area index (LAI, this is a measure of leaf cover), the position of the leaf in the canopy, and the measurement time. The latter is of influence, because the angle of solar radiation changes over the day. Leaves can be exposed to solar radiation in the early morning and late afternoon and be in the shade during mid-day. The leaf orientation might have influence on the solar radiation a sunny leaf receives. When the leaf in the northern hemisphere is facing south it will receive more direct sunlight, than the north facing leaves.

Water availability also influence the stomatal conductance. When there is enough soil moisture available the irradiance has the highest influence on the opening and closure of the stomata. However, when the plant experiences water stress, the stomata can close earlier, in order to maintain a certain critical leaf water potential (Turner, 1974). One of the hormones that is influenced by water stress is abscisic acid (ABA), which increases in the roots and leaves when the water potential decreases. It is suggested that ABA signals pass along hydraulic signals which influences the stomatal opening (Araújo et al., 2011; Lambers et al., 2008; Yan et al., 2017). Zhao et al. (2015) observed a higher stomatal density, but a smaller stomatal size and opening was observed for situation with low soil water potential. For corn, substantial stomatal closure can be observed at leaf water potential values of -2 MPa (Nobel, 2009). (Turner, 1974) observed stomatal closure at -1.7 MPa.

Under water stressed conditions, plants can be divided into two categories: isohydric species and anisohydric species. Isohydric plants keep a constant ψ_l throughout the day by reducing the stomatal conductance. For anisohydric species, ψ_l is more variable. The stomata stay open for a long period, which can lead to a decrease ψ_l. Early stomatal closure will take place in anisohydric plants to precent dehydration, but is not sufficient to maintain a constant ψ_l (Sade et al., 2012; Tardieu and Simonneau, 1998). Corn (*Zea mays L.*) is an isohydric species. From study by Tardieu and Simonneau (1998) it followed that ψ_l during the day stayed at a minimal value of -1.7 MPa, even when water stress was applied. In the well-watered condition, the stomatal conductance was highest between 8:00 and 10:00, but stayed high for the rest of the day. In the water stressed conditions, the stomatal conductance decreased after 9:00 and remained low for the rest of the day.

The CO_2 concentration in the ambient air influences the opening of the stomata (Kim et al., 2010; Lambers et al., 2008; Vanaja et al., 2011). The stomatal opening increases as the CO_2 concentration in the ambient air is low. To ensure sufficient CO_2 for photosynthesis, and when the concentration CO_2 is high, more stomata will close (Araújo et al., 2011). When a plant experiences water stress, the plant becomes more sensitive to the CO_2 concentration. A stronger response is observed stomatal closure as a result of low concentrations in case of water stress (Vanaja et al., 2011). When comparing results from different sites or days, it is important take into account that results can be influenced by a difference in the CO_2 concentration in the ambient air.

The relative humidity also influences the opening of the stomata. Raschke (1970) found that the stomata of corn are more closed in dry air. Besides the opening of the stomata, the VPD between the leaf and the air influences the stomatal conductance. The VPD is determined by the relative humidity of the ambient air, and therefore influenced by the air temperature, the leaf temperature and the wind speed. As the relative humidity of the ambient air is low, the VPD is high which leads to a high transpiration if the stomata are opened. When there is dew formation on the canopy, the VDP is low, as the RH of the ambient air is high. Therefore there is little transpiration while there is dew on the canopy Ben-Asher et al. (2010). As mentioned, not only the relative humidity influences de VPD, but also the temperature of the air and the leaf. As the temperature increases, the air can contain more water vapour. Even so, as the leaf temperatures increases, the water vapour inside the leaf increases as well. This increase in water vapour inside the leaf happens generally faster than the increase of water vapour in the ambient air, which leads to an increase in VPD (Lambers et al., 2008). However, transpiration cools the leaf, as the phase change of water that takes place requires energy. When the stomata close as a result of water shortage, the leaf temperature increases. Besides the influence of leaf temperature on the VPD, it is also used to detect water stress in crops. The wind speed influence the stomatal conductance by influencing the ambient air around the leaf.

In summary, the leaf water potential and stomatal conductance are controlled by various different environmental factors and by the feedback loop between the stomatal conductance and leaf water potential. It is therefore of great importance to take the the environmental conditions, such as solar radiation, relative humidity, precipitation and if possible soil water potential, into account when analysing the leaf water potential and stomatal conductance.

3
Methods

This chapter gives a description of the used materials and methods regarding the data collection. Measurements of the stomatal conductance and the leaf water potential will be the main focus of this research. Other measurements which has been executed describes the meteorological conditions, hydrological conditions, and, to some extent, the plant hydraulics at the study field. All measurements are described in this section, with an emphasis on the stomatal conductance and the leaf water potential.

3.1. Site description

The field campaign where the data was collected took place on the Plant Science Research & Education Unit of the University of Florida, located in Citra, FL, see Figure 3.1. The climate in Citra is a humid subtropical climate (Cfa) according to the Köppen-Geiger climate classification (Peel et al., 2007). The annual precipitation is 1143 $mm/year$ (over period 2001-2017 FAWN data), of which most falls from June to September. October to May are the drier months, with much lower precipitation. The reference evaporation, which is determined with the Penman-Monteith (Allen et al., 1998), annually cycle with a trough in January and a peak in June and July. With the combination of low precipitation and high evaporation, the months March, April and May are most suitable for field experiments on water stress, see Figure 3.2. The soil of the field consists for 95% of sand (Bongiovanni et al., 2015b; **?**), which allows a high infiltration rate. The high infiltration rate in combination with the high evaporation rates, high solar radiation, low amounts of rainfall, makes it relatively easy to impose water stress on the corn on this location.

The original planning was to start the project in the beginning or March. However, the late arrival of key instruments for the project caused a delay of a month. Therefore, measurements were conducted from 13 April 2018 to 18 June 2018. The weather conditions are on average less optimal to apply water stress from mid-May onward, as frequent rainfall events in the afternoon are common. In 2018, the months May and June appeared to be much wetter than the expected seasonal precipitation. The delay of the project, in combination with high precipitation, meant that the original research aim of comparing a water stressed with a well-watered situation, would not be achieved. This research takes place under well-watered conditions. Establishing a protocol for the measurements, and the lessons learned from the experiments are of high importance for future experiments, and that not only dependent on a water stressed situation.

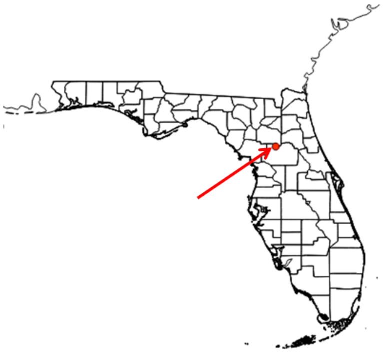


Figure 3.1: Field location, in Citra, Florida, USA

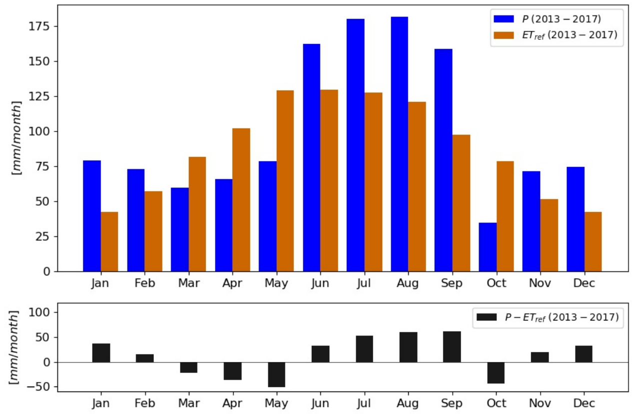


Figure 3.2: (a) Average monthly precipitation ($P[mm/month]$) and reference evaporation ($ET_{ref}[mm/month]$), (b) and the average monthly effective rainfall ($P-ET_{ref}[mm/month]$) at Citra for the period 2013-2017.

3.2. Field set-up

The field experiments have been executed as part of a larger field campaign and experiments of the University of Florida. The field campaign was designed in such a way that, when desired, a different irrigation regime could be applied to each half of the field. By doing so, water stress could be imposed on part one half the field. During this field campaign, the same irrigation regime is applied to both sides of the field.

In figure 3.3 an overview of the experimental set-up is shown. In the northern part of the field, a radar of Delft University of Technology was installed. Just south of the radar footprint the soil moisture, soil water potential, sap flow and leaf wetness sensors were installed. South of that, 8 plants in the same row were selected for the stomatal conductance measurements. The same plants were measured throughout the whole season. The plants for the leaf water potential were collected from 2 areas, one on the west side of the field, and one on the east side. On both sides of the field, there were two locations for the sample collection for the biomass and the vegetation moisture content measurements.

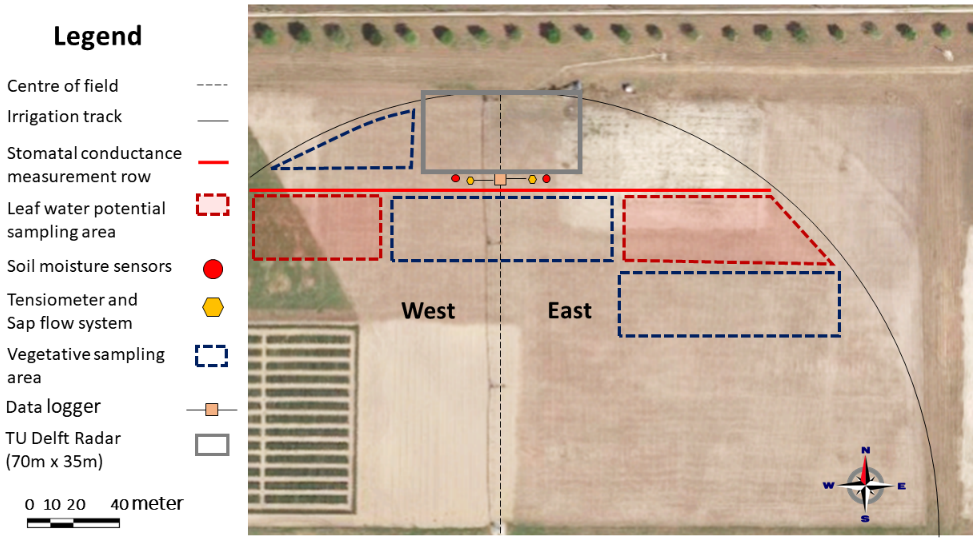


Figure 3.3: Overview of the field campaign set-up, including areas that contain the footprints of the radars.

3.3. Meteorological data

3.3.1. Weather station data

The meteorological data was obtained from a flux tower from Florida Automated Weather Network (FAWN)[1] at the Plant Science Research & Education Unit. The tower is located 500 m east of the measured field, as indicated in figure 3.3. From this flux tower, every 15 minutes data is obtained on soil temperature [$°C$] at 10 cm depth, air temperature [$°C$] at a height of 60cm, 2m and 10m, relative humidity [%], rainfall [inches], solar radiation [W/m^2], wind speed [MpH] at 10m , and the wind direction [°]. The dew point temperature at 2m [$°C$] has been calculated with the air temperature [$°C$] at 2m and the relative humidity [%]. The wet bulb temperature [$°C$] has been calculated using the Newton-Raphson algorithm. Both have an interval of 15 minutes. The daily reference evaporation [inch/day] was calculated with the Penman-Monteith Allen et al. (1998).

3.3.2. Leaf surface wetness data

Decagon leaf wetness sensors, were installed on a pole between the plants. The first sensors were installed 24 April 2018 at 7 cm height. The heights of the sensors have been adjusted during the growing season, to represent the leaf wetness at different height of the canopy, see 3.1.

Table 3.1: Placement heights of the different leaf surface wetness sensors.

Date	Sensor 1	Sensor 2	Sensor 3
24 April - 4 May	7 cm	7 cm	-
5 May - 8 May	10 cm	20 cm	-
8 May - 11 May	10 cm	20 cm	30 cm
12 May - 28 May	25 cm	50 cm	75 cm
29 May - 1 June	40 cm	80 cm	105 cm
2 June - 14 June	40 cm	80 cm	120 cm

[1] `https://fawn.ifas.ufl.edu/`

3.4. Soil data

At the Plant Science Research & Education Unit of the University of Florida the composition of the top layer of the soil consists of approximately 94% sand, 2% silt, 3% clay and 2% organic matter, where the percentages are given on a weight basis. At the depth of 40 cm, a different soil layer was encountered, see Figure 3.5. In earlier field work at the same field, an increase of approximately 1% in sand, and a decrease of approximately 0.5% silt and 1% organic matter was observed, at depth of 40cm and downwards Bongiovanni et al. (2015a;b).

3.4.1. Root zone soil moisture

The root zone soil moisture is measured by Vermunt et al. (2019) with 10 calibrated EC-5 sensors. In the same row, two pits were made, one on each side of the field, see Figure 3.3 and Figure 3.4. The sensors were installed in each pit at 5, 10, 20 40 and 80 cm depth. The root zone moisture was measured throughout the season with an interval of 15 minutes.

3.4.2. Soil water potential

Two T4e pressure transducer tensiometers were installed to measure the soil water potential. One on each side of the field, 3 rows south of the soil moisture sensors, see Figure 3.3. The tensiometer was installed under an angle of 40 $^\circ C$, the middle of the ceramic cup was at a depth of 20 cm. These measurements were conducted by Vermunt et al. (2019).

Figure 3.4: Installation of the soil moisture sensors. Photo by author.

Figure 3.5: Different compositions of sand observed at soil moisture sensor installation. Photo by author.

3.5. Corn

Sweet corn, type BSS0977 ATTRIBUTE 100M, was planted on 13 April 2018. With a growing season of 72 days, the corn was harvested on Monday 18 June 2018. The plant density was determined on 6.6 $plants/m^2$, with a plant spacing of 7 $plants/m$, and row spacing of 0.925m. The only limitation for plant growth was competition between plants. Figure 3.6 shows the numbering of the leaves, and the different heights measured during the field campaign.

3.5.1. Growth stages

The growing season of a corn plant consist of many stages, which can be divided into two major parts: (1) the vegetative stage, and (2) the reproductive stage. During the vegetative stage, the plant grows in height and the leaves develop. The tasseling is the last phase of the vegetative stage. During the reproductive stage the fruit develops and ripens. If a plant experience water stress, the period in which this occurs influences the further development of the plant. Water stress during the vegetative stage reduces the plant height, the leaf area development and grain yiel to 18.6-26.2% (Cakir, 2004; Mi et al., 2018). During the reproductive stage, water stress has a larger impact, which can reduce the grain yield with 41.6-46.6% (Mi et al., 2018) or even 66-93% Cakir (2004).

Three times a week, the growth stage of the corn was determined with the use of the BBCH staging Manual (Earth Observation and Research Branch Team, 2011). In Appendix A shows the description of each stage. When determining the BBCH-stage, only completely unfolded leaves are counted. This is also called the "droopy" leaf method. The vegetative stage is from BBCH 10 - BBCH 59. The reproductive stage is from BBCH 61 - BBCH 99.

3.5.2. Leaf area index

The leaf area index (LAI) is the one-sided leaf area of plants per unit of ground surface area (m^2/m^2). Once a week, canopy geometry measurements were conducted on four to eight plants, including the maximum leaf length (l_{max}) and width (w_{max}) for all leaves. The assumption was made that a leaf had an ellipse shape, see Equation 3.1. For each plant, the LAI was calculated by use of recorded measurements in combination with the plant density. The sum of leaf area of all leaves was multiplied by the plant density, as shown in Equation 3.2.

$$A_{leaf} = l_{max} * w_{max} * \pi \tag{3.1}$$

$$LAI = \Sigma A_{leaf} * plant\ density \tag{3.2}$$

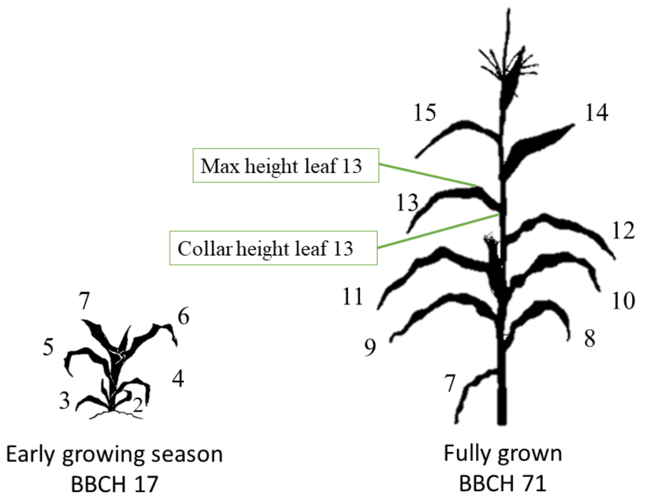


Figure 3.6: Leaf numbers at different moments in the growing season.

3.6. Stomatal conductance measurements

3.6.1. Materials

The stomatal conductance is a measure of the water vapor exiting the stomata in $mmol/m^2s$. The abaxial (bottom surface) stomatal conductance was measured with the hand-held Decagon Leaf Porometer, model SC-1, Figure 3.7. These were non-destructive measurements and the same plants could therefore be measured during the season.

The leaf porometer measures the rate at which water vapor passes through the stomata into the atmosphere. Measurements can be taken when the temperature is 5 - 40 $°C$ and with relative humidity of 1-100%, when the desiccant chamber is used. The accuracy is 10% for a stomatal conductance between 0 - 1,000 $mmol/m^2s$. If the stomatal conductance is above 1,000 $mmol/m^2s$, the measurements are less accurate. The hand-held Leaf Porometer has to be calibrated in the beginning of each day. An extra calibration needs to take place as soon as the difference in temperature exceeds 15 $°C$.

The minimum time for stomata to close as a response to low humidity next to the leaf is 2 minutes. A measurement of the leaf porometer takes 30 seconds, therefore the used instrument has no effects on the stomatal conductance itself. However, it is important that the leaf is not touched at the measured spot, nor should two measurements be taken at the same spot soon after each other. One measurement took approximately 2 minutes, including 30 seconds of taking the measurement, writing down the desired information and equilibrating the sensor for the next measurement.

The differences of humidity and temperatures, measured within the porometer, was used in order to determine the stomatal conductance. The porometer is equipped with two humidity sensors, the first one is located just below the leaf, and second one at the bottom of the sensor, just above the desiccant chamber, as can be seen in figure 3.8. The humidity is measured at the two sensors, Inside the leaf the relative humidity reaches 100%. The temperature is measured at the two sensors, and it is assumed that the temperature of the leaf is equal to the first sensor. The distance between the leaf surface (d_1) and between the two sensors (d_2) are known to be respectively 3.35 mm and 11.43 mm. With this information, and the assumption that the vapor flux is constant between any two nodes, the stomatal conductance can be calculated with the following equation:

$$g_s = \frac{\hat{\rho} D_{vapor}[h_{r1}e_s(T_{a1}) - h_{r2}e_s(T_{a2})]}{[e_s(T_{a1})(1-h_{r1})]d_2 - [h_{r1}e_s(T_{a1}) - h_{r2}e_s(T_{a2})]d_1} \tag{3.3}$$

where:

$$\hat{\rho} D_{ref} = (44.6)(2.12 * 10^{-5}) \left(\frac{T}{273.15}\right)^{0.75} \tag{3.4}$$

$$e_s(T_a) = 0.611 * exp\left(\frac{17.502T}{T + 240.97}\right) \tag{3.5}$$

with:
g_s = stomatal conductance of the leaf surface
$\hat{\rho}$ = molar density of air
D_{vapor} = diffusivity of water vapor
h_{r1} = relative humidity in humidity sensor 1, just below the leaf surface
h_{r2} = relative humidity in humidity sensor 2, above desiccant chamber
$e_s(T_{a1})$ = saturated vapor pressure at the air temperature of sensor 1 in $°C$
$e_s(T_{a2})$ = saturated vapor pressure at the air temperature of sensor 2 in $°C$
d_1 = the distance between the leaf surface and the first humidity sensor (3.35 mm)
d_2 = the distance between the first and the second humidity sensor (11.43 mm)

The measurement becomes more accurate as the difference in humidity between sensor 1 and sensor 2 increases. Adding the desiccant in the desiccant chamber below sensor 2, decreases the relative humidity at that point to near zero, which results in a steep gradient in humidity and therefore a more accurate measurement. More detailed information on how the leaf porometer works, and how the equation for the stomatal conductance is built up, can be found in the Decagon leaf porometer Operator's manual [2].

[2] `http://manuals.decagon.com/Manuals/10711_Leaf%20Porometer_Web.pdf`

Figure 3.7: Decagon leaf porometer SC-1

Preliminary measurements were taken on 24 April 2018 throughout the day on different locations on the leaf and on different plants. From this information the measurement protocol was adjusted where needed. on 26 April, the stomatal conductance measurements started.

To capture the diurnal difference of a plant, the same plants were measured. Between 26 April and 4 May 2018, the measurements were done on the same 6 plants, which were different from the plants for the rest of the season. On 7 May, the official field layout was set, and the final plants for the stomatal conductance measurements were selected and flagged. All selected plants were located in the same row, see Figure 3.3. Of these plants, 4 were on the west side of the field, and 4 on the east side. At this stage, the plants were 30 cm high and the shading did not play an important role yet. With the selection of the plants, the orientation of the leaves was taken into account. For each plant selected on the west side, a plant with similar leaf orientation was selected on the east side.

The plants were chosen to be at least a meter apart from each other. This was done for two reasons: First, in the beginning of the growing season, all plants are small and of equal height. As the plants grow, some areas in the field might have smaller plants, or very large plants. By selecting the plants at different locations in the row, the variation of the field is better represented. Second, there are changes that the plants will be damaged during the season, due to pests or due to torn or broken leaves. By not selecting plants directly next to each other, a damaged plant can be replaced by a neighbouring non-damaged plant with similar orientation and shading of the leaves. It occurred that the wrong leaves were measured on one plant on the west side of the field after a certain day. This plant was excluded from further analysis.

3.6.2. Stomatal conductance measurement protocol

Measurements were taken every 3 hours, 3 times a week, starting at 10:30. Earlier measurements were not possible because of dew formation on the leaves. Moreover, rain prevented several measurements. As most rain fell in the afternoon, limited measurements were taken after 14:00. The measured times on the different days can be found in Table 3.2.

Each day, the Decagon Leaf Porometer was calibrated before the first measurement. For accurate measurements and calibration, the Porometer sensor, and the USP Purified Water used for the calibration, need to be in thermal equilibrium with the environment of the field, which takes up to 10 minutes. After reaching the thermal equilibrium the Leaf Porometer was be calibrated according to the instructions in the Operator's Manual.

During each measurement, 3 to 4 leaves were measured, mostly on the same side of the plant. The focus leaves were the high leaves and the leaves around the ear, as the most dynamic behaviour is expected to take place in those leaves. Therefore, the highest unfolded leaf was measured, the second leaf below it, and so on, see Figure 3.6. As the plant was growing, the measured leaves shifted up. An overview of the measured leaves on each measurement day is given in Table 3.2.

To take the measurement, the porometer was carefully, and with minimal touching of the leaf, placed on one of the sides of the leaf at approximately 2/3 of the leaf length from the tip on an undamaged spot, see Figure 3.9 This is often the higher part of the leaf and is easily detectable on the different leaves. This ensures a comparable position on the leaf between the different measurements. Since the solar radiation influences the photosynthesis, and thus the opening of the stomata, the measurement point was picked such that shading at that point was equal to the shading of the majority of the leaf. The leaves were measured from lowest to highest location, to prevent shading the lower leaves while measuring the top leaves.

During the measurement, several conditions were noted, such as: weather or not the leaf was shaded, the shading at the actually measured point on the leaf and if there was a cloud limiting the solar radiation during the measurement. At the first measurement round of the day, the maximum leaf height was measured for each of the measured leaves. Appendix B shows the information collected per measurement.

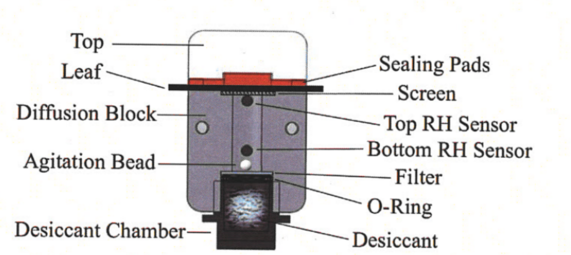


Figure 3.8: Sensor head of the Decagon Leaf Porometer SC-1. (picture from the Decagon Leaf porometer Operator's manual)

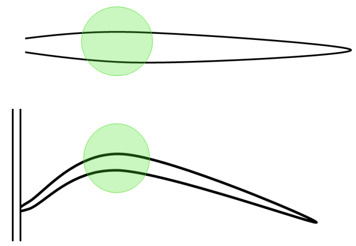


Figure 3.9: Placement of the porometer on the leaf, in top view (top) and side view (bottom).

Table 3.2: Overview of the stomatal conductance measurements, with the measurement times and measured leaves per measurement day.

Date	**Measurement times**	**Measured leaves**			
26 April 2018	10:00, 13:30, 17:30	Leaf 1	Leaf 2	Leaf 3	Leaf 4
02 May 2018	10:00, 12:15	Leaf 2	Leaf 3	Leaf 4	Leaf 5
04 May 2018	10:50, 14:30	Leaf 3	Leaf 4	Leaf 5	
07 May 2018	10:30	Leaf 3	Leaf 5	Leaf 7	
09 May 2018	14:50, 20:15	Leaf 3	Leaf 5	Leaf 7	
11 May 2018	10:15, 12:00, 14:00	Leaf 3	Leaf 5	Leaf 7	
16 May 2018	10:30	Leaf 5	Leaf 7	Leaf 9	
18 May 2018	10:00, 12:30	Leaf 5	Leaf 7	Leaf 9	Leaf 11
23 May 2018	10:30, 13:20, 15:15, 17:20	Leaf 5	Leaf 7	Leaf 9	Leaf 11
24 May 2018	10:50, 13:15	Leaf 5	Leaf 7	Leaf 9	Leaf 11
25 May 2018	10:30, 13:50	Leaf 5	Leaf 7	Leaf 9	Leaf 11
29 May 2018	11:00	Leaf 5	Leaf 9	Leaf 13	
01 June 2018	12:20, 14:10	Leaf 7	Leaf 9	Leaf 11	Leaf 13
04 June 2018	11:00, 12:30, 15:40	Leaf 7	Leaf 9	Leaf 11	Leaf 13
11 June 2018	10:40, 12:20, 13:40, 15:40, 17:50, 19:30	Leaf 7	Leaf 9	Leaf 11	Leaf 13

3.7. Leaf water potential measurements

3.7.1. Materials

The leaf water potential was measured using a PMS-600 pressure chamber (Corvallis, Oregon, USA) with a Grass Compression Gland Sealing System, as shown in Figure 3.10a. The pressure chamber has a diameter of 6.3 cm and a depth of 12.7 cm.

The pressure chamber measures the leaf water potential in the following way: A leaf is cut at a certain length, so it still fits in the pressure chamber without the sample being damaged. The sample is placed in the pressure chamber, and the lid is carefully closed. After the chamber is sealed, the pressure is slowly increased until water appears at the cut surface of the leaf. The amount of pressure applied to reach that point is a measure of the stress the leaf is experiencing. The water stress the plant is experiencing is high whenever a high pressure needs to be applied. The applied pressure was measured in bar or PSI and later converted to MPa (1 MPa = 10 bar ≈ 145 PSI). The reading accuracy was 0.5 PSI, which equals approximately 0.0034 MPa.

The leaf water potential was measured on 3 plants per sample area as indicated in figure 3.3. For each measurement day, the plants were selected from the same row, within the sample areas. The selected plants were of representative height, had no damages on the leaves that were measured, and as little damages as possible on the rest of the plant. For each day, the same leaf numbers were measured for the leaf water potential as for the stomatal conductance. Table 3.3 shows the leaf numbers measured on each day.

(a) Experiment set-up.

(b) Photo of a measurement with the pressure chamber.

Figure 3.10: The PMS-600 pressure chamber.

3.7.2. Leaf water potential measurement protocol

The first measurements for the leaf water potential were done using the first protocol: First, a representative plant was chosen. Then, the collar height and maximum leaf height were measured for the leaves of interest and the leaf orientation, and if possible the shading. Appendix B shows the field form used. Finally, the 3 or 4 leaves of interest were cut at maximum length and put in a plastic bag to be taken to the measurement instrument, located 5 minutes' drive from the field. There, the leaves were measured with use of the pressure chamber. After the measurement, a plant on the other side of the field was measured in the same way, and so on, until three plants from each field side were measured. In this way, the difference between the two sides of the field was minimal and the average of the three measured plants on each side could be compared. It is desired to measure the leaves as soon as possible after cutting them. It is recommended to take the measurements within 20 minutes after cutting.The time limit of 20 minutes is used for difference between cutting and measuring the corn samples in this experiment. With the above described protocol, the time between cutting the leaves and conducting the actual measurements exceeded 20 minutes. This was even the plants were still small and the measurements were, therefore, easier and faster compared to later in the growing season. To ensure more reliable data, the protocol had to be changed. This was done on the 9th of May.

For the second protocol, plants were taken from one side of the field at a time. Three adjacent plants were all together removed from the soil, including roots and surrounding soil. The plants were selected to be field-representative and to have as little damages as possible. Of each plant, the leaf water potential of 3 or 4 leaves was measured. The measured leaves were at the same height as those measured for the stomatal conductance measurements on that day (Table 3.3). After measuring the first batch of plants, three adjacent plants were removed from the other side of the field. When the measurements started on the west side, all measurements of that day started on that side. The first measured field side switched every measurement day to get an indication of possible different pre-dawn conditions between the two field sides.

At the measurement location, the plants were measured one by one. Before the start of each series of measurements, the safety valve was checked as described in t he pressure chamber manual. The leaves of a plant were cut at the same time and placed in separate plastic bags. A paper towel was placed in the plastic bags to limit water loss due to transpiration. The sample length of the leaves changes over the season. Until the 16th of May the whole leaf is measured in the pressure chamber, and after that, the longer leaves are cut at 40, 45 or 50 cm from the tip, to fit into the pressure chamber. The length of the leaf that would fit in the chamber without damaging the leaf was 30 cm to 50 cm, depending on the total length of the leaf and therefore the flexibility of the nerve at that distance from the tip. The exact length of the leaf sample from tip to cut for each leaf is given in Table 3.3.

To take the measurement, a wet paper towel was inserted in the pressure chamber, to ensure sufficient humidity surrounding the leaf during the measurement and prevent transpiration. The sides of the leaf were pulled back and the nerve with approximately 1 cm of leaf on each side, was inserted in the glass gland seal. The seal was closed carefully, to prevent breaking the nerve. The leaf was carefully rolled up into the pressure chamber and the lid closed. When the leaf was inserted and the lid closed, the pressure in the chamber was increased with a rate of 0.05 MPa per second. A head light and x16 hand lens were used to observe the cut surface, see Figure 3.10b. The leaf water potential was reached just before water bubbles appeared on the cut surface. At that moment, the valve was closed and the applied pressure was read from the instrument. A clear cut of the leaf improves the visibility of the cut surface for water bubbles.

Three times a week, measurements were done at 6:00 and 8:00, and once a week at 18:00 and 20:00. The evening measurements were done on the same days as the destructive measurements for biomass and vegetation moisture content. The lowest leaf water potential is reached between 12:00 and 15:00. Leaf water potential measurements at mid-day were limited since they were conflicting with the stomatal conductance measurements. A mid-day measurement was done in the beginning of the season on the 9th of May and at the end of the growing season on the 8th of June.

Table 3.3: Overview of the leaf water potential measurements per measurement day, with the measurement times and measured leaves with (the sample length in cm measured from the leaf tip in cm).

Date	Measurement times	Measured leaves (sample length cm)			
04 May 2018	7:00, 8:40	Leaf 3 (max)	Leaf 4 (max)	Leaf 5 (max)	
07 May 2018	6:40, 8:20	Leaf 3 (max)	Leaf 5 (max)	Leaf 7 (max)	
09 May 2018	6:00, 7:40, 12:30, 13:40, 18:00, 19:30	Leaf 3 (max)	Leaf 5 (max)	Leaf 7 (max)	
11 May 2018	6:10, 7:40	Leaf 3 (max)	Leaf 5 (max)	Leaf 7 (max)	
14 May 2018	6:10, 8:00	Leaf 5 (max)	Leaf 7 (max)	Leaf 9 (max)	
16 May 2018	6:30, 7:50, 18:50, 20:30	Leaf 5 (max)	Leaf 7 (45)	Leaf 9 (45)	
18 May 2018	6:20, 7:50	Leaf 5 (max)	Leaf 7 (45)	Leaf 9 (45)	
21 May 2018	6:10, 8:20	Leaf 5 (1/2 max)	Leaf 7 (45)	Leaf 9 (45)	Leaf 11 (45)
23 May 2018	6:20, 8:30, 18:40, 20:20	Leaf 5 (1/2 max)	Leaf 7 (40)	Leaf 9 (45)	Leaf 11 (45)
25 May 2018	6:20, 8:10	Leaf 5 (1/2 max)	Leaf 7 (40)	Leaf 9 (45)	Leaf 11 (45)
29 May 2018	6:20, 8:20	Leaf 5 (1/2 max)	Leaf 9 (45)	Leaf 13 (45)	
30 May 2018	6:20, 8:10	Leaf 5 (1/2 max)	Leaf 9 (45)	Leaf 13 (40)	
01 June 2018	6:20, 9:20	Leaf 7 (40)	Leaf 9 (50)	Leaf 11 (50)	Leaf 13 (40)
04 June 2018	6:30, 9:20, 18:20, 20:30	Leaf 7 (40)	Leaf 9 (50)	Leaf 11 (50)	Leaf 13 (40)
06 June 2018	6:00, 6:20	Leaf 7 (40)	Leaf 9 (50)	Leaf 11 (50)	Leaf 13 (40)
08 June 2018	6:10, 6:20, 13:00, 13:10, 18:20, 18:40	Leaf 7 (40)	Leaf 9 (50)	Leaf 11 (50)	Leaf 13 (40)
11 June 2018	6:10, 9:00	Leaf 7 (40)	Leaf 9 (50)	Leaf 11 (50)	Leaf 13 (40)

3.8. Sap flow

The sap flow was monitored with a Dynagage Flow32-1K Sap Flow system on four representative plants, two on each side of the field, see Figure 3.3. The first two sensors were installed on the 19th of May, as the plant had the minimum required diameter for these sensors of 15 mm. From the 1st of June onward, data is collected with all 4 sensors.

4 Results and Discussion

In this chapter the results of the measurements are given. The weather, and in particular the precipitation, had a large influence on the rest of the measurements. Therefore, the observations in meteorological data are analysed first. This is followed by the most important results regarding the soil data, and by an overview of the corn development. After this, the stomatal conductance measurements are analysed, both on a seasonal time scale and for several days. For the leaf water potential the results are also given and analysed on a seasonal and diurnal time scale. The last results that are analysed is from the sap flow. Finally, a synthesis reflects on the results of the different measurements.

4.1. Meteorology

4.1.1. Weather station data

In the Methods, Chapter 3, it was explained that the field campaign location was chosen because of the possibility to apply water stress to part of the field. The generally low precipitation in the months March-May, in combination with high evaporation makes this possible. However, in 2018, there was an exceptional high amount of rainfall. Figure 4.1 shows the monthly precipitation and evaporation for 2018 next to the average monthly precipitation and evaporation in 2013-2017. In April and May of 2018, the precipitation was respectively 170 mm and 205 mm, which is more than twice the 5-year average precipitation of 66 mm for April and 79 mm for May. The reference evaporation was similar in April and in May slightly lower than the 5-year average. This lead to a precipitation minus reference evaporation of 71 mm in April and 95 mm in May, compared to a five year average of respectively -36 mm and -50 mm. The large amount of precipitation had great influence on the field campaign and this research. The planting date had to be delayed because part of the equipment did not arrive on time. On top of that delay, the planting date was delayed further by a week because the field was too wet to plant the corn. Because of this delay, the majority of the data was collected in May and June. On average, the precipitation increases after mid-May. Under average circumstances, it might still be possible for the corn to experience water stress in these months, as the evaporative demand is high, see Figure 4.1. In 2018, the precipitation was so high, that no water stress could be imposed during the growing season. The research therefore focuses only on well watered conditions.

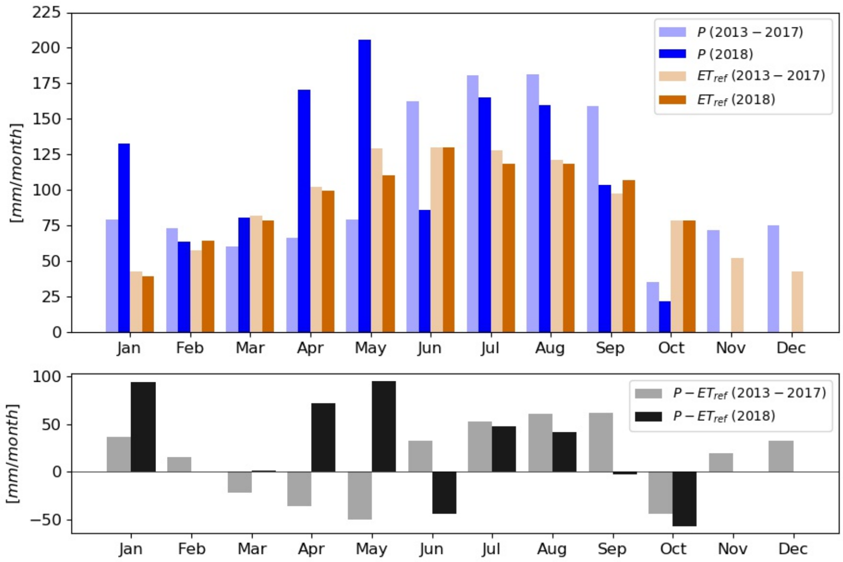


Figure 4.1: (a) the monthly precipitation ($P[mm/month]$) and reference evaporation ($ET_{ref}[mm/month]$) in 2018 compared to the 5 year monthly averages over the period 2013-2017, (b) and the monthly effective rainfall ($P - ET_{ref}[mm/month]$) for 2018 compared to the5 year average monthly effective rainfall for 2013-2017 at Citra, FL.

Figure 4.2 shows the meteorological data measured at the FAWN weather station and calculated by FAWN[1] between 24 April and 19 June. In the beginning of the growing season, from April 24 to May 13, there was hardly any rain and irrigation was applied to ensure water availability for the initial growth of the corn. The soil and air temperature showed a relative constant diurnal pattern. The reference evaporation has a value of approximately 4 mm/day. The solar radiation and the relative humidity show also a clear diurnal cycle, with low relative humidity at the end of the day of 20-50%. Over the season, the time between sunrise and sun set increased. In the beginning of the growing season, dawn is at 6:45 and sunset at 20:45. At the end of the growing season dawn is at 6:15 and sunset at 20:45.

On 14 May, the summer rains started with a rainy period, until 22 May. Less variation in the diurnal air temperature was observed during this time. The solar radiation was lower, because of heavy cloud cover on several days. The relative humidity was higher, with minimum values of 60% in the afternoon of 19 May, and a less constant diurnal pattern is observed. The wind speed had a more variable behaviour compared to the previous days. Because of these differences, the reference evaporation, calculated with the Penman-Monteith equation, is also lower on the days with rainfall. Between 22 - 27 May, there was limited rainfall, and similar, but less distinct, diurnal patterns as before 14 May were observed for the air temperature, solar radiation and relative humidity.

On 27 May, there was another heavy rainfall event, followed by rainfall events on the following days. The air- and soil temperature during the day stayed low, limited solar radiation was observed and the relative humidity stayed high. After the 1st of June, the rainfall events were less extreme, and the meteorological observations show similar clear diurnal patterns as in the beginning of the growing season. On 6 June and 10 June, smaller rainfall events took place. The influence of these events on the observed data is limited.

The stomatal conductance depends highly on the solar radiation, but also on the relative humidity, air temperature and wind speed. The two periods with rain and heavy cloud cover should be taken into account when analysing the data, and when comparing the results to similar data collected in the future.

[1]https://fawn.ifas.ufl.edu

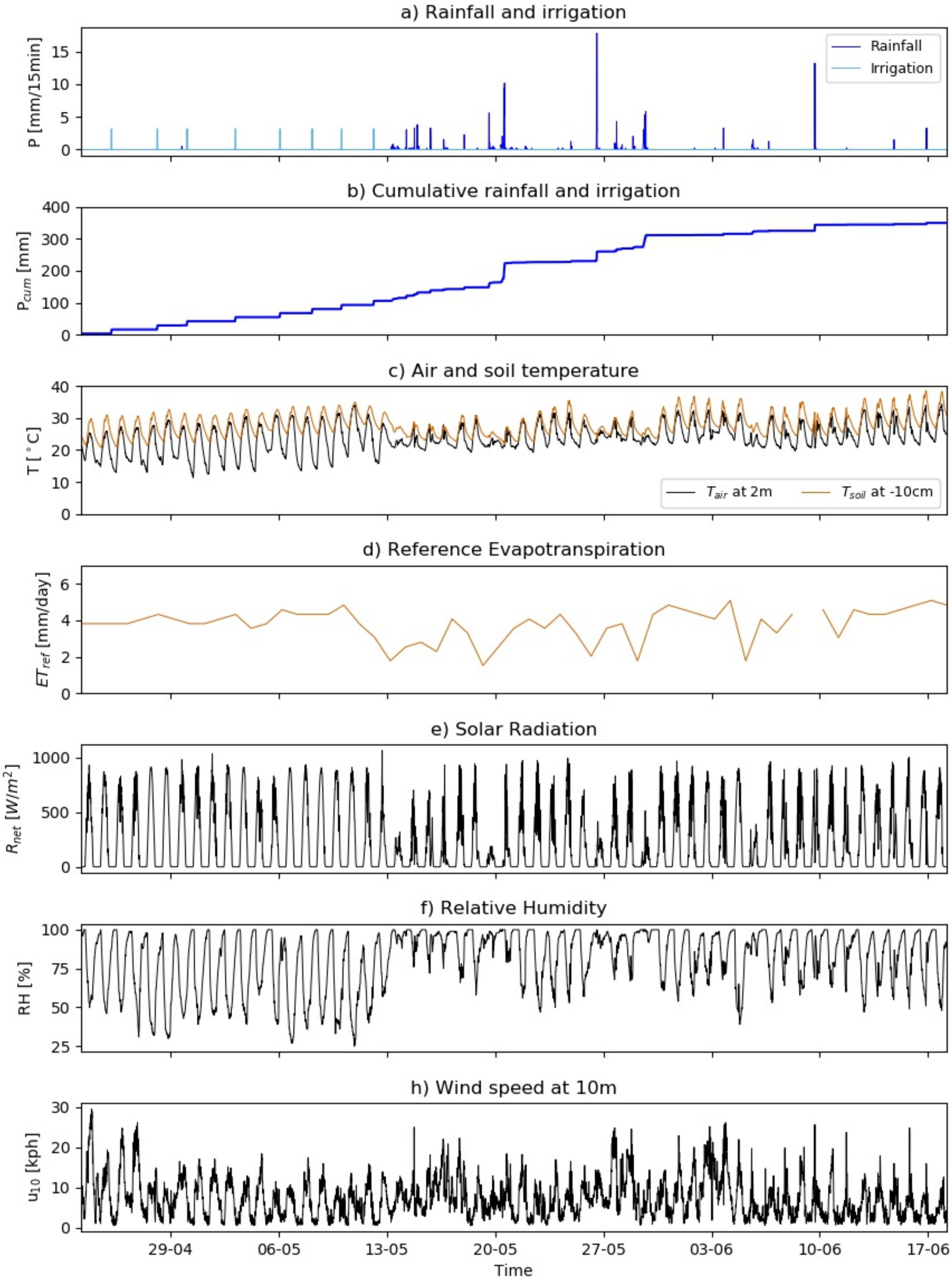


Figure 4.2: Meteorological data collected at the FAWN weather station at the educational farm in Citra, FL.

4.1.2. Irrigation

Irrigation of the entire field took place on several days, see table 4.1. The original plan was to apply moderate water stress to one half of the field by reducing the irrigation from 14 May onward. However, due to a high amount of rainfall, sufficient water was available, and no irrigation was needed after 12 May.

Table 4.1: Dates and amounts of irrigation applied to the experiment field

Date	Amount (mm)
26 April 2018	12.7
28 April 2018	12.7
30 April 2018	12.7
4 May 2018	12.7
6 May 2018	12.7
8 May 2018	12.7
10 May 2018	12.7
12 May 2018	12.7

4.1.3. Leaf surface wetness

The leaf wetness was observed by Vermunt et al. (2019) at 3 sensors at different heights . When analysing the data, a distinction is made between leaf wetness from dew, irrigation and precipitation. Dew was observed on the leaves until approximately 10:00 in the morning during the complete growing season. When irrigation has taken place overnight the leaves tend to be wet until the same time, or about an hour longer compared to the days with no irrigation. Before the rainfall events, starting on 14 May, the dew remained longest on the leaves that were low in the canopy, and it disappeared first on the leaves higher in the canopy. After the rain events, the middle sensor detected dew the longest. In the first two weeks of June, there were short afternoon rains frequently. This could also be observed from the leaf wetness sensors. There was some variation between the sensors at different heights. The dew was observed longest in the lower sensor. After rainfall events, the surface of sensor 1 stayed wet for a longer time. The second sensor had the least wet surface. These results are of importance for this research for two reasons: the stomatal conductance measurements can only be taken on dry leaves, and second, the stomatal conductance is determined by the water vapour pressure deficit (VPD). The VPD is smaller when the air around the leaf is humid.

4.2. Soil data

4.2.1. Root zone soil moisture

Figure 4.3 shows the root zone weighted soil moisture (RZSM) over a depth of 1 m. An increase in soil moisture was observed after a rainfall or irrigation event. A diurnal pattern in soil moisture content above 20 cm depth was observed, with a decrease during the day, caused by evaporation. Each day between 5:00 and 11:00, a small increase, or constant value, in the soil moisture was observed at 5 and 10 cm depth. This was caused by dew infiltrating into the soil. The soil moisture at 80 cm depth increased after the precipitation events on 16, 21, 28 and 30 May. In both, the West and East pit, the soil moisture content was lowest at 5 cm depth. In the west pit, the soil moisture at 10 cm and 40 cm depth was less than that of the east pit, while the soil moisture at 20 cm was higher in the west pit. The difference can be due to a different exact position of the sensors, or a slightly different composition of the surrounding soil.

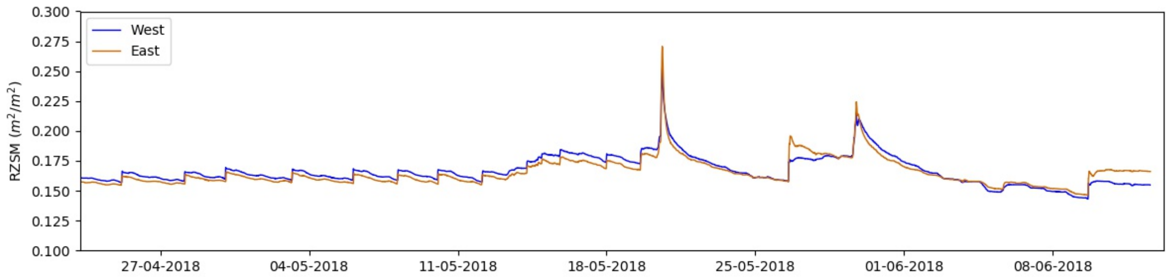


Figure 4.3: Root zone weighted soil moisture over 1 m depth measured from 24 April to 13 June in the East pit and the West pit.

4.2.2. Soil water potential

Figure 4.4 shows the observed soil water potential on the west and east side of the field. During most of the season, ψ_s was high for both sides as the soil was almost saturated. On May 21, when a heavy rainfall event occurred, the soil was saturated and ψ_s reached values of 0 MPa. After 21 May, ψ_s decreased. On the east sensor, the largest decrease was observed. On 27 and 30 May precipitation events increased the soil water potential again up to nearly 0 MPa on both sides. Between 1 June and 5 June, ψ_s starts to decrease, with a period of low soil water potential from 5 June to 10 June. Again, the east sensor measured a significant lower ψ_s. This can be related to sensor and measurement errors or due to a difference in the surrounding soil. The lower soil water potential in the west side is corresponding to the lower soil moisture content observed in the west pit, see Figure 4.3. The soil water potential increases again at 10 June, after a precipitation event.

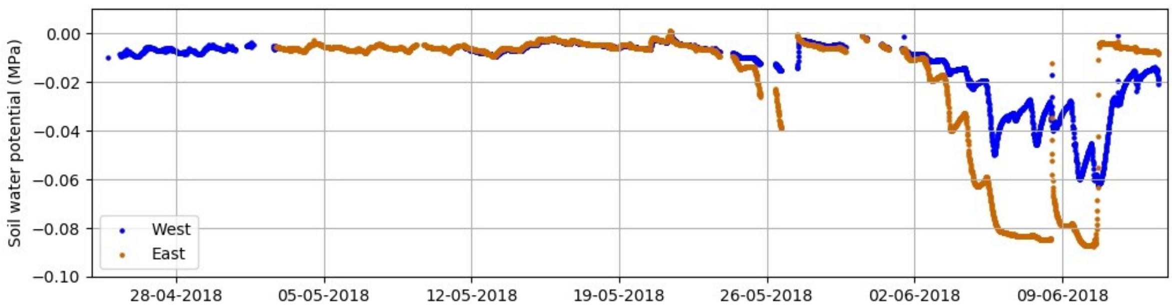


Figure 4.4: Soil water potential measured on the east side of the field and the west side of the field form 24 April until 14 June. Derived from Vermunt et al. (2019)

4.3. Corn Development

Figure 4.5 shows how the corn field developed during the growing season. Until BBCH 59 (31 May), the corn is in the vegetative stage. From BBCH 61 (1 June), the flowering and fruit development starts and the plant is in the reproductive stage. Figure 4.6 shows the development of the plant indicated in maximum plant height, the height of the lowest leaf and the leaf area index (LAI).

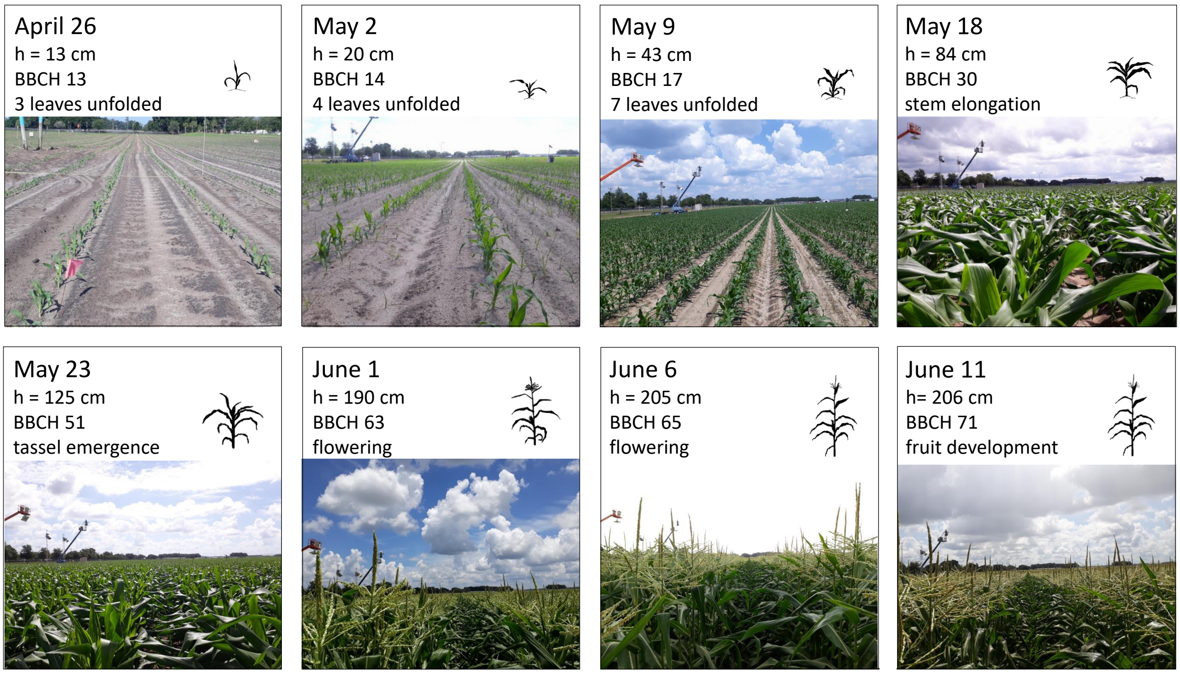


Figure 4.5: Overview of growing season of the sweet corn

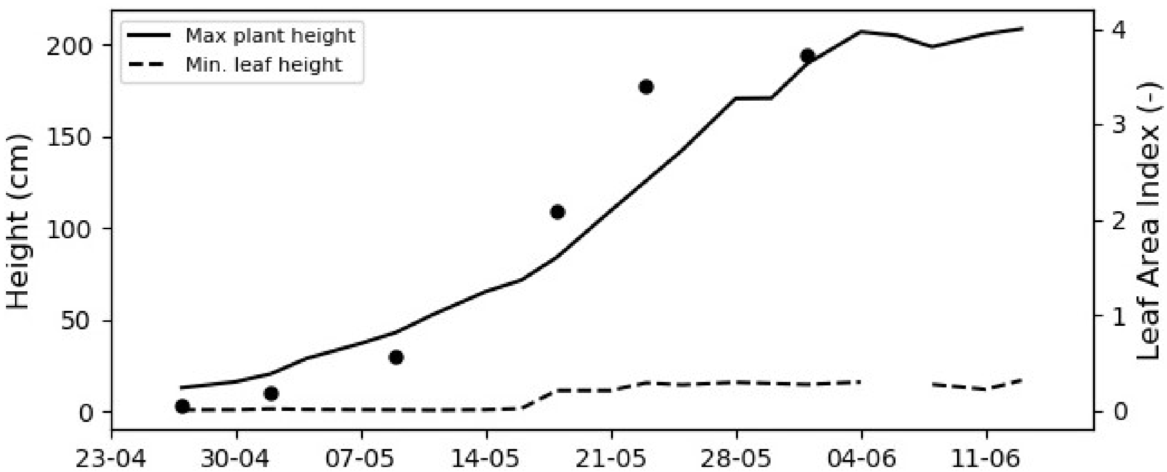


Figure 4.6: The maximum plant height, minimum leaf height and LAI of the corn over the growing season. Data derived from Vermunt et al. (2019).

4.4. Stomatal conductance

The stomatal conductance was ideally measured three times a week, from the morning until the early afternoon. No measurements were possible on wet leaves. As dew covered the canopy until 10 A.M., the measurements started at 10:30 in the morning. The rainfall that took place after 14 May resulted in wet leaves. This prevented several measurements from taking place.

In this section, the results of the stomatal conductance measurements are analysed and discussed. First the results on the seasonal conductance and the observed variation over height during the season are analysed. This is followed by the results on the diurnal variation in time and height. After that, the changes over the growing season in the diurnal cycle of the stomatal conductance are analysed. Last, is a brief discussion of the measurements.

4.4.1. Seasonal variation in stomatal conductance

An overview of all data collected over the growing season on the stomatal conductance per leaf number is shown in Figure 4.7. The measurements done on sunlit leaves are represented by a yellow dot, the mainly shaded leaves by a blue dot. A black circle around the measurement point indicates that the leaf was partially sunlit, partially shaded. The color within the black circle indicates the shading at which the measurement was done. From this figure, it is visible that sunny leaves have a higher stomatal conductance, with values up to 1200 $mmol/m^2s$, and in general a wider spread than the shaded leaves, which have values mainly below 500 $mmol/m^2s$.

In the beginning of the season, all leaves are exposed to solar radiation. There is no limit in stomatal conductance by solar radiation. As the plant grows, the lower leaves become partially shaded, as is observed on 4 May. The stomatal conductance is lower in the beginning of the season, and increases at 11 May. On 26 April, the plant is in BBCH 13. This means that the plant is at the end of the phase in which the seed is its primary nutrient source and at the beginning of the photosynthetic process (Seminis, 2015). This might be the reason for the lower stomatal conductance observed in the beginning of the season.

From the 11th of May onward, the lower leaves are shaded and leaves start to be partially shaded. On 18 May, the plants begin to reach to those of the next row. Between 18 and 23 May, full effective ground cover is reached. From this moment, the lower part of the canopy stays shaded, and a large part stays partially shaded. Between 23 and 25 May, many measurements were done. Tables with the shading of each leaf number during the different growing stages can be found in Appendix C.

In Figure 4.8 the stomatal conductance per leaf is shown. The yellow dots are the measurements done on predominantly sunny leaves, the blue dots on leaves in the shade. On 26 April, 23 May and 11 June, most measurements were done. Between 11 May and 23 May, and between 28 May and 1 June, wet leaves caused by rainfall limited the measurements.

As the plant grew, the lower leaves became more shaded. This started at 11 May (BBCH 18) for leaf 5, at 23 May (BBCH 51) for leaf 7 and leaf 9, and at 1 June (BBCH 61) for leaf 11. Eventually, the lowest leaves became completely shaded, which was on 23 May for leaf 5 and on 1 June for leaf 7. Last, the lowest leaves dried out and died as senescence took place. The stomatal conductance of the higher leaves was not limited by the blockage of solar radiation by other leaves. These leaves showed therefore the highest range in stomatal conductance. Most transpiration losses took place from these leaves. A low stomatal conductance was observed in the beginning of the season, with values of max 800 $mmol/m^2s$. It increased from 11 May onward, and stayed around a value of maximal 1200 $mmol/m^2s$ for the rest of the season. The measurements in the sun, had a stomatal conductance in the range of $250-1200 mmol/m^2s$. The leaves that were completely in the shade have clearly a lower stomatal conductance in the range $0-500 mmol/m^2s$.

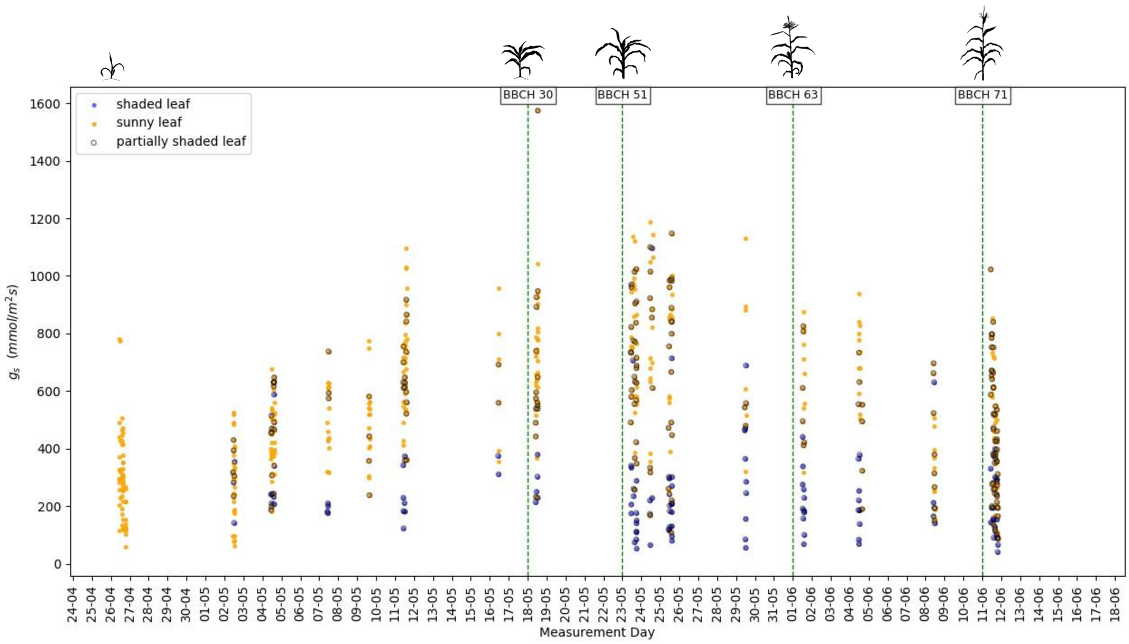


Figure 4.7: Overview of the measured stomatal conductances. A distinction is made between sunny leaves (yellow) and shaded leaves (blue). Leaves that were partially sunny, partially shaded have a black circle around the measurement point. Measurements were there was heavy cloud cover, or those done after sunset were excluded.

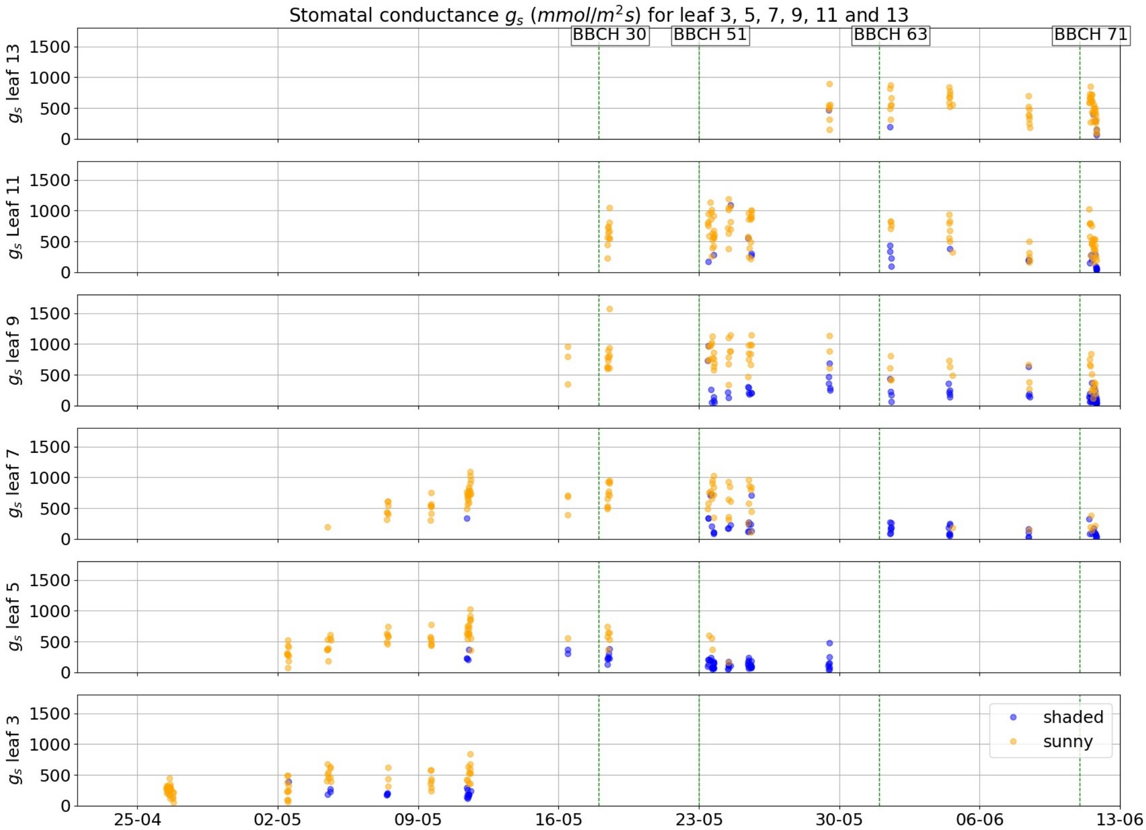


Figure 4.8: Overview of all stomatal conductance measurements per leaf. A distinction is made between sunny leaves (yellow) and shaded leaves (blue).

4.4.2. Diurnal variation in stomatal conductance

The stomatal conductance was measured max. three days a week. The measurements started from 10:00 in the morning until the early afternoon. Ideally, once a week the stomatal conductance was measured from the morning until the late afternoon to capture the diurnal behaviour of the stomata. By doing this weekly, changes in the diurnal behaviour over the season could be analysed. However, because of rainfall, on only four days three or more measurements were done. This was on 26 April, 11 May, 23 May and 11 June. On 26 April and 11 June stomatal closure was captured. The stomatal conductance observations, together with the measured solar radiation are shown in Figure 4.9-4.12. Appendix D shows the diurnal plots for the other measurement days. On all days similar patterns and behaviour was observed as on the days shown below.

As cloud cover influences the solar radiation received by a leaf, the measurements taken with clouds are indicated by a grey color in Figure 4.9-4.12. If the cloud cover was so heavy and long lasting that the actual shading of the measurement point could not be seen with the eye, the measurement point was indicated as being shaded. When combining the collected information on shading from the stomatal conductance measurements with the solar radiation from FAWN, it should be taken into account that the FAWN station is located 500 m east of the field. The exact timing of the clouds can therefore deviate from that observed in the field. In the data, a fast respond to cloud cover is observed on multiple days. The stomatal conductance decreases as a heavy cloud passes over which halves the solar radiation. This was observed on 23 May. Complete stomatal closure because of cloud cover was only observed in the afternoon of 4 June.

Besides cloud cover, the leaf orientation and leaf temperature might influence the stomatal conductance, as described in Chapter 2. Appendix E shows the stomatal conductance for the leaf orientation of each measured leaf early in the growing season. No clear influence of the leaf orientation was observed in the stages BBCH 12-18 (26 April - 11 May). After BBCH 18, the shading by other leaves played a major role in the variation of the stomatal conductance. Appendix F shows figures with the leaf temperature, stomatal conductance, air temperature and solar radiation. From this analysis it became clear the the leaf temperature is highly affected by the solar radiation. Another observation is that the leaf temperature is similar at a specific time of the day for all measured leaves, regardless of the stomatal conductance.

26 April Figure 4.9 shows the results of measurements conducted on 26 April per measured leaf, in combination with the solar radiation observed at the FAWN station. Besides some light cloud cover around 13:30, there was no blockage of solar radiation. As the plant was 12 cm high, with a maximum of four leaves, shading caused by leaves did not play a role. The results show a clear diurnal cycle. Although the measurements started at 10:00 and the early opening of the stomata was not captured, an increase in stomatal conductance is observed between 10:00 and 11:00. Leaf 2 had the highest stomatal conductance through out the day. During the first two measurement rounds, the stomatal conductance stayed mostly within the range 200-500 $mmol/m^2s$. No high increase in stomatal conductance was observed in the afternoon, when the solar radiation and temperature were highest, and the relative humidity lowest. After 18:00 a decrease in stomatal conductance is observed, caused by the decrease in solar radiation.

11 May Figure 4.10 shows the observed stomatal conductance on 11 May. The plant has grown to a maximum height of 50 cm. Measurements were done between 10:00 and 15:00, so no full diurnal cycle was measured. The stomatal conductance increases between 10:00 until the maximum measured values are reached at 15:00. A clear difference between the measured leaves is observed. Leaf 3 has the lowest stomatal conductance. Leaf 3 is mostly shaded early in the morning, and becomes sunlit in the afternoon. The stomatal conductance for the shaded leaves is low. As the leaves become sunny, the stomatal conductance increases. In leaf 5, a clear difference in stomatal conductance between the shaded and sunny leaves is observed. The shaded leaves have a stomatal conductance below 500 $mmol/m^2s$, sunny leaves have a stomatal conductance of roughly 500-1000 $mmol/m^2s$. The majority of the leaves is partially sunny, partially shaded by higher leaves. It is observed that the stomatal conductance of a partially shaded leaf measured at a sunny spot is not lower than the stomatal conductance of a full sunny leaf. Nor does a measurement at a shaded spot of a partially shaded leaf have a higher stomatal conductance than a completely shaded leaf. Leaf 7 is the third highest leaf. As leaf 9 is still small, leaf 7 is fully exposed to the sun. Leaf 7 has the highest stomatal conductance.

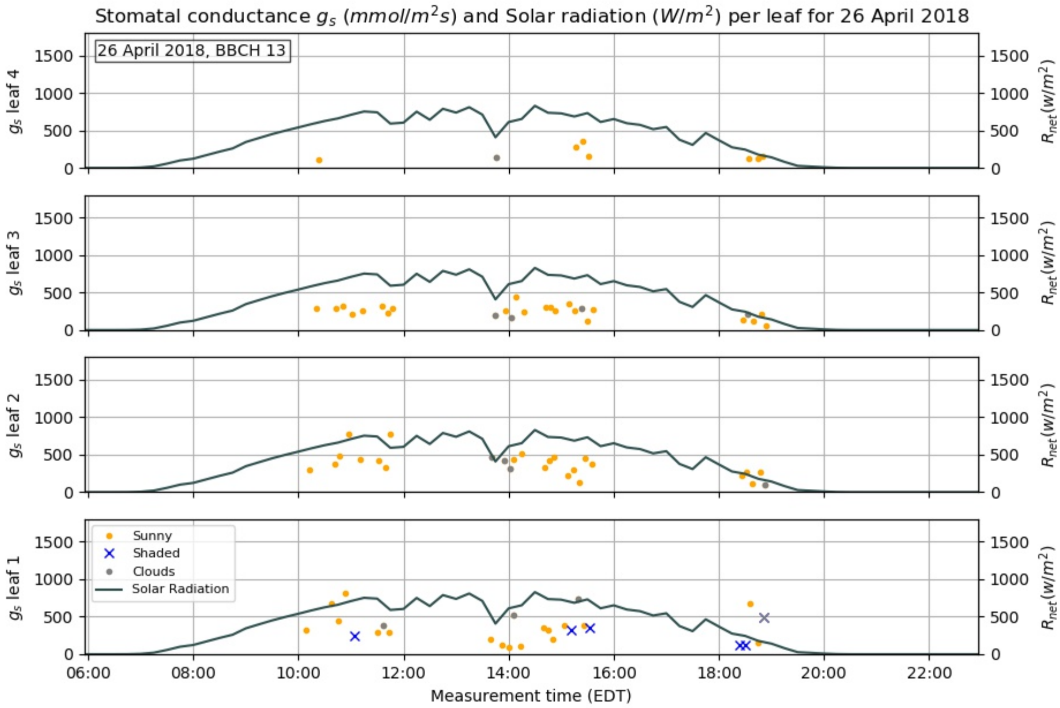


Figure 4.9: The stomatal conductance observations for the different measured leaves and the solar radiation measured at the FAWN station on 26 April.

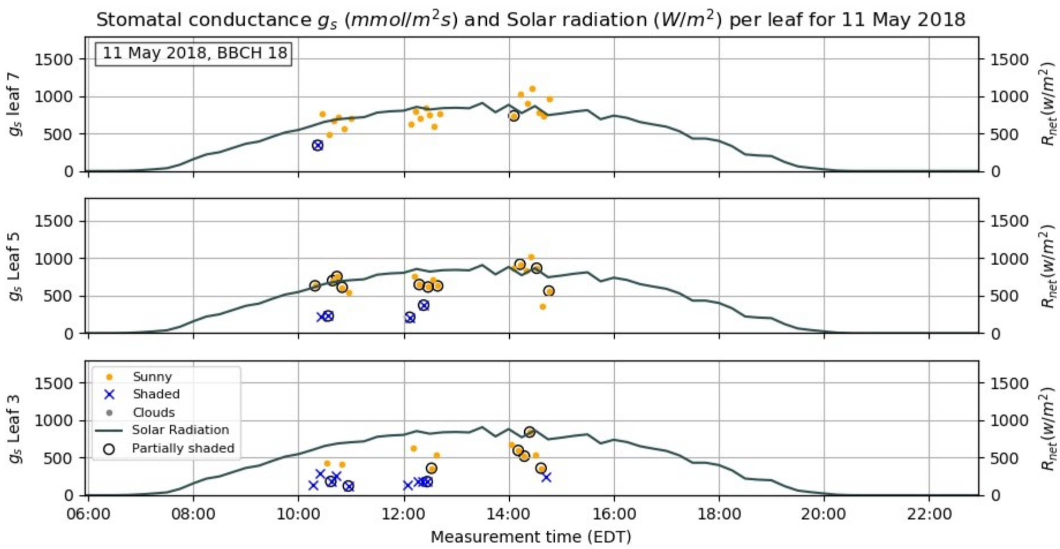


Figure 4.10: The stomatal conductance observations for the different measured leaves and the solar radiation measured at the FAWN station on 11 May.

23 May Figure 4.11 shows the results for 23 May. An increase in stomatal conductance until 12:00 was observed for all leaves. In the late afternoon, still high values of stomatal conductance were observed but less measurements had a high stomatal conductance. The diurnal cycle could not fully be measured as the leaf water potential measurements had to be done. At several moments of the day, clouds blocked the majority of the solar radiation. This is not only observed in the solar radiation data, but also in the stomatal conductance data.

A closure in of the stomata is observed during cloud cover. At 12:00, the response to the cloud cover is clearly observed in the last two measurements done for leaf 9 and leaf 7, and at 14:00 for leaf 7, 9 and 11. Stomata have a reaction time of 5 to 20 minutes (Nobel, 2009). This delay in response time is visible in leaf 11 at 12:00: The leaves are already shaded by clouds, but the stomatal conductance has still the same order of magnitude as when there was no cloud cover. The solar radiation observed at the FAWN station gives useful insight in the change in the solar radiation and the response of the stomata. When a deep trough is observed in the solar radiation, the stomatal conductance is very low. However, the observed solar radiation does not match exactly with the observed cloud cover in the field. At 14:00, a trough was observed in the solar radiation at FAWN. For both leaf 9 and leaf 11, in-between the measurements taken under cloud cover, some measurements were taken in the sun, and with a high stomatal conductance. At 15:30, limited solar radiation is observed at FAWN, however, no measurements with cloud cover were done in the field at that time.

The difference in stomatal conductance over height is more distinct compared to the previous discussed days. Leaf 5 is mostly shaded and has a low stomatal conductance throughout the day. Except three measurements that were made on sunny sports, all measurements were made on shaded spots. These had results of 50 - 235 $mmol/m^2s$. The variation over the day was minimal. As clouds were observed, the already low stomatal conductance, decreased even more. Leaf 7 is mostly shaded, by higher leaves and/or clouds. At the end of the afternoon, as the solar radiation reaches the field from a lower angle, more of the leaf 7 receive solar radiation. This results in a small increase in measured stomatal conductance at the end of the day. Leaf 9 has more sunny leaves and higher stomatal conductances than the lower leaves. At 11:00 the high values of 100 $mmol/m^2s$ are reached by partially shaded leaves. The stomatal conductance stays around 1000 $mmol/m^2s$ until 16:00, leaves with cloud cover are not taken into account. Between 17:00 and 18:00, the stomatal conductance is lower for all leaves, but no stomatal closure of the sunny leaves is observed. For leaf 11, the stomatal conductance is comparable to that of leaf 9. For most leaves, only clouds limit the solar radiation. The responds to clouds is high, as described above. When those measurements are not taken into account, a slight increase in stomatal conductance is observed between 10:00 and 12:00. The stomatal conductance stayed at a value around 1000 $mmol/m^2$ until 15:00. At the end of the day, the spread in stomatal conductance was with 500-1000 $mmol/m^2s$, larger than observed earlier in the day on sunny leaves. Most measurements between 17:00 and 18:00 had a stomatal conductance between 500-600 $mmol/m^2s$.

11 June The diurnal measurements of 11 June are shown in Figure 4.12. The highest stomatal conductance is observed during the first and second measurement round, between 10:00 and 15:00. At 16:00, the measured stomatal conductances were lower, around 400-600 $mmol/m^2s$ for sunny leaves, and below 500 $mmol/m^2$ for shaded leaves or under clouded circumstance. At 18:00, a further decrease in stomatal conductance was observed, until stomatal closure took place after 20:00. In the variation over height, the same patterns are observed as on 23 May. Considerably less measurements were done for leaf 7, as this leaf was dying as a result of senescence.

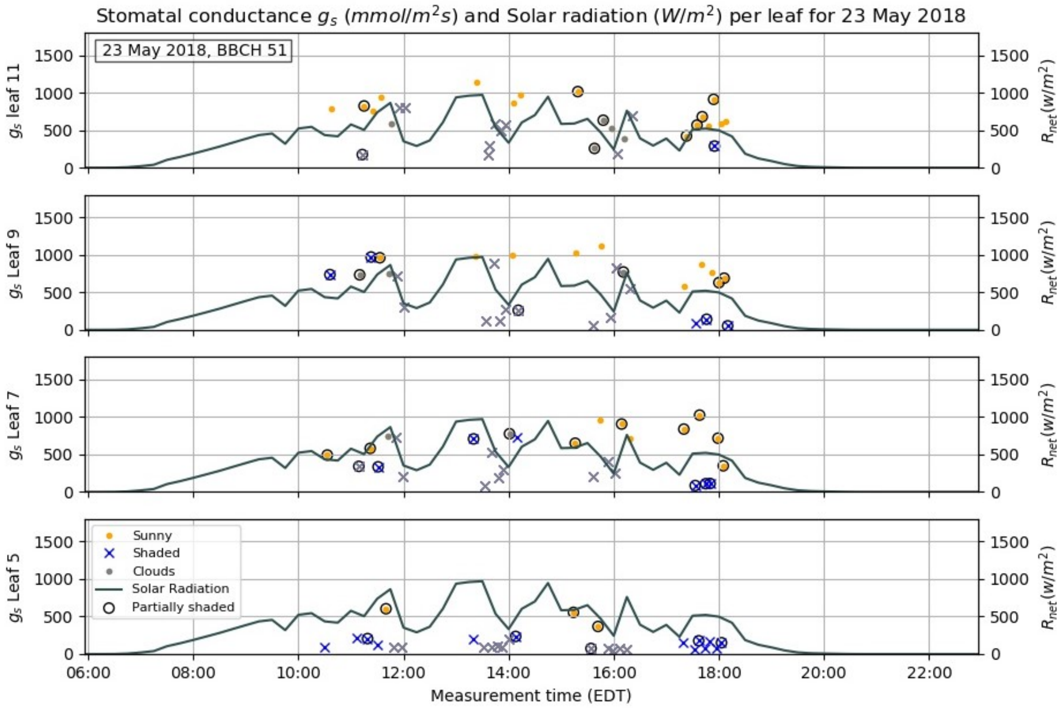


Figure 4.11: The stomatal conductance observations for the different measured leaves and the solar radiation measured at the FAWN station on 23 May.

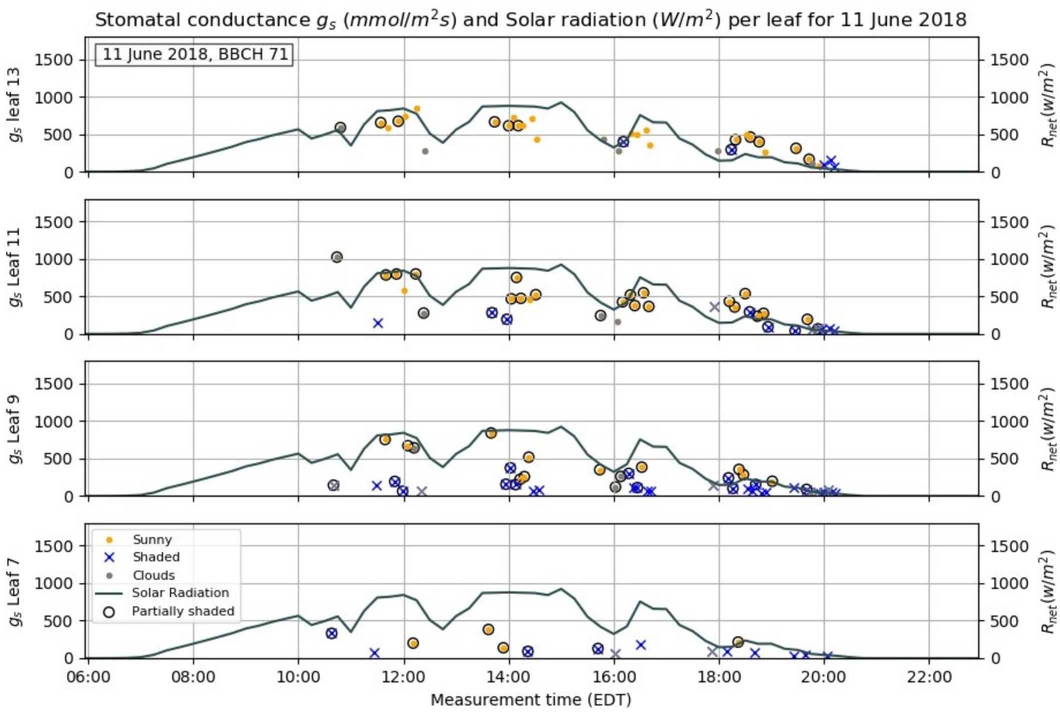


Figure 4.12: The stomatal conductance observations for the different measured leaves and the solar radiation measured at the FAWN station on 11 June.

4.4.3. Changes in diurnal cycle of stomatal conductance of the season

From collected data no hard quantitative conclusions can be drawn. For this, more complete diurnal cycles should have been observed, with a higher density, preferably under comparable weather circumstances for optimal comparison. However, seasonal patterns and trends can be distinguished, by combining the observed diurnal cycles with the rest of the collected data. To do so, the growing season is split into three parts:

- Beginning of the vegetative stage: 26 April - 18 May (BBCH 13 - 19): In this period, the leaf development takes place and LAI has a steep increase.
- End of the vegetative stage: 19 May - 31 May (BBCH 30 - 59): In these stages, the last three leaves will develop, stem elongation takes place and doubles the length of the corn plant, and the tassel emerges and separates.
- Reproductive stage: 1 June - 11 June (BBCH 61 - 71): In this part of the growing season, the plant is fully grown, flowering takes place and the ears start to emerge and develop.

Beginning of the vegetative stage: BBCH 13 - 19 An increase in the stomatal conductance was observed as the plant grows. In the beginning of the season, the lower leaves had a higher stomatal conductance. As the plant grew, higher stomatal conductance were measured at the top leaves. The shading of the leaves changes most in this part of the growing season. At BBCH 13 (26 April), all leaves were equally sunny. After BBCH 15 (4 May), shading caused by leaf cover was observed for leaf 3. At BBCH 18 (11 May), a difference in stomatal conductance over height was observed, influenced by the shading by other leaves. The shading of the leaves was depended on the measurement time. Leaf 3 was often shaded, leaf 5 was shaded at certain times, and the top leaves of the canopy were sunny. The shaded leaves had a low stomatal conductance, and the lower sunny leaves had a lower stomatal conductance than the upper leaves, on 11 May.

End of the vegetative stage: BBCH 30 - 59 The differences in canopy is less than in the beginning of the vegetative stage. On 23 May, full ground cover was reached (LAI > 3), resulting in shading of the ground and the lower leaves. The variation over height observed at BBCH 18 continued and shifted up in the canopy as the plant continued to grow. On 18 May, leaf 5 was partly shaded and partly sunlit during the day. Leaf 7, 9 and 11 had no limitation in solar radiation by higher leaves. On the 23 and 25 May, leaf 5 was shaded for most of the measurements, and leaf 7 was shaded half of the time. This lead to a lower stomatal conductance for these leaves. Leaf 9 moves from being a complete sunny leaf, to a partially shaded leaf, but with high stomatal conductances. The stomatal conductance was highest in leaf 9 and leaf 11. The observed stomatal conductances in this period were higher than in the beginning of the vegetative stage. Only on 23 May, measurements were done at the end of the day. On this day, as shown in Figure 4.11 the stomatal conductance stayed at around 1000 $mmool/m^2s$ until the solar radiation had decreased and the stomata closed.

Reproductive stage: BBCH 61 - 71 As the plant is fully grown, the shading of the different leaves hardly changed within the period. Only few measurements were made in this period, and these were highly influenced by cloud cover. Senescence started, and after 4 June, leaf 7 was dying at several plants. The measured sunny leaves had a lower stomatal conductance than during the late vegetative stages. With the exception of one measurement, no stomatal conductance above 1000 $mmol/m^2$ was observed.

From this analysis, a few point become clear:

- The main difference between the stages and over season is the shading over the height of the plant. This is closely related to the BBCH-stages and the LAI.
- In the beginning of the growing season the stomatal conductance is low, as the plant grows it increases.
- The highest stomatal conductances were observed during the late vegetative stages, where the last leaves are emerging, and the stem elongation and tasselling takes place.
- In the late vegetative stages, a clear variation in stomatal conductance over height was observed.
- In the reproductive stages, there is a clear variation over height which does not change any more.
- The highest stomatal conductance observations in the reproductive stages were lower than during the late vegetative stages.

4.5. Leaf water potential

In this section the results of the leaf water potential measurements are analysed. First, the different factors that influence the leaf water potential and the measurements are discussed. Then, the seasonal variation of the leaf water potential is analysed. This is followed by an analysis and discussion of the diurnal measurements at 9 May, 23 June and 8 June. Finally, an analysis of the changes in the diurnally observed leaf water potential over the growing season is given. This analysis is done by combining the discussed diurnal observations with the rest of the data. Not all observed data will be shown in this section. Appendix G shows results on the variation in ψ_l between the observed leaves. The observations in variation over height for individual days are discussed in this chapter.

4.5.1. Seasonal variation in Leaf water potential

Figure 4.13 shows the measured leaf water potential for the different leaves at 6:00, and the measured soil water potential in the east and the west pit. Between 8 May and 4 June, the plants were well watered and ψ_l at 6:00 is similar with average values between 0.1 MPa and 0.25 MPa. The ψ_l at 6:00 is nearly equal over the height of the plant for that period.

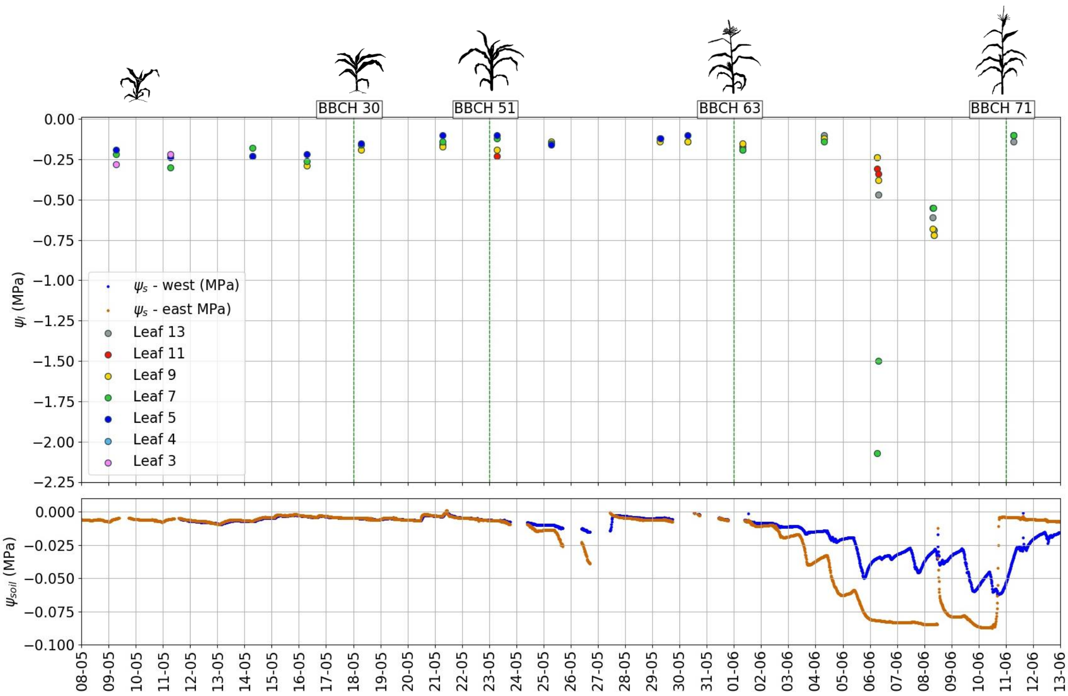


Figure 4.13: Leaf water potential at 6:00 per leaf number and soil water potential. The soil water potential data, presented in the lower figure, is derived from Vermunt et al. (2019).

From the theory described in Chapter 2, one could expect that ψ_s and ψ_l at pre-dawn to be close to each other, as the stomata are closed during the night. However, in Figure 4.13 a difference in pre-dawn ψ_s and ψ_l between 0.12 MPa (18 May) and 0.64 MPa (8 June) is visible. This difference is called the pre-dawn disequilibrium (PDD). Night time transpiration is the most relevant factor causing this (Donovan et al., 2003; Kangur et al., 2017). The night time transpiration takes place as stomata are partially opened in combination with the VPD of ambient air. Kangur et al. (2017) found that the PDD was large after drier nights, but close to zero after cool and humid nights. The same pattern can be observed from the data. In Figure 4.2f, a lower relative humidity during the days, but also at night, is observed in the first part of May. During this time, ψ_l at pre-dawn was lower than it was for the rest of May, where the relative humidity was higher.

The decrease in ψ_s between 4 June and 10 June is visible in the ψ_l measurements at 6:00. On 6 and 8 June, a lower ψ_l was observed at 6:00 than previously. The spread between the different leaves was larger than in the previous days. On 6 June, leaf 7 shows average ψ_l of -1.50 MPa and -2.05 MPa, since some of the measured leaf 7 were starting to senescence. This lead to a much lower ψ_l. For a senescing leaf ψ_l was -3.1 MPa. On 8 June and 11 June, leaf 7 was only measured when the senescing had not started.

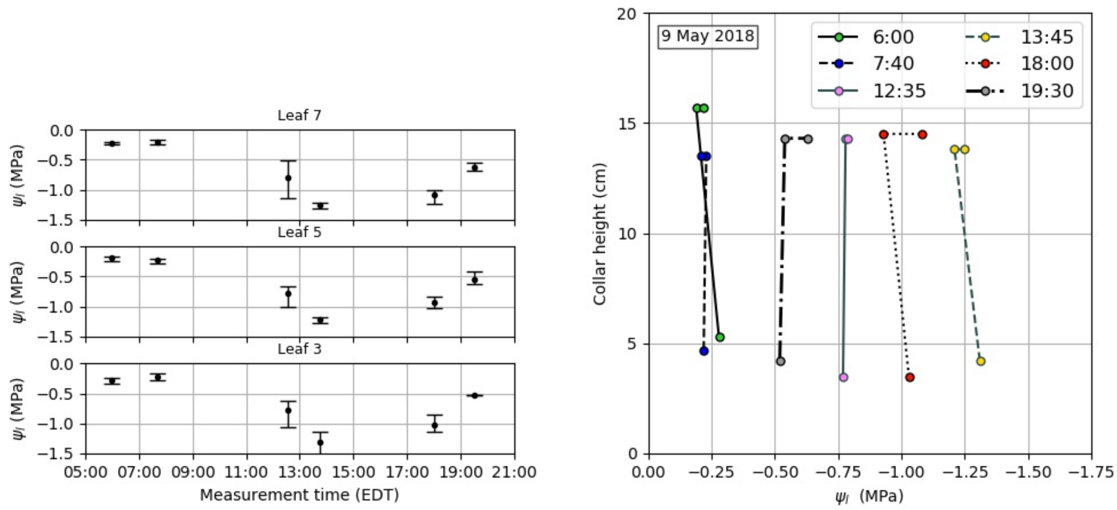
From literature it followed that the photosynthesis of corn is affected as the leaf water potential drops below -1.70 MPa (Boyer, 1970; Nobel, 2009; Turner, 1974). Leaf rolling, which is a control mechanism for corn when water stress occurred, was observed by Baret et al. (2018) when the leaf water potential dropped below -1 MPa. From this references, and the observed plant behaviour, we can not conclude that water stress caused by low soil water potential was observed during this fieldwork. There is a chance that there was light water stress in the morning of 10 June, as the soil water potential reached the lowest values on that day. However, as no measurements were taken on 10 June, this is unknown.

The rainfall event on 10 June increased the ψ_s. The ψ_l at 6:00 had also increased on 11 June. The observed ψ_l had values around -0.20 MPa, and all leaves had a comparable ψ_l. This shows that the decrease in ψ_l observed on the 6th and 8th of June are related to the water availability, and not to the starting senescence.

4.5.2. Diurnal variation in Leaf water potential

On five days the leaf water potential was measured at pre-dawn in the morning, and in the evening. On the first and the last measurement day, 9 May and 8 June, the mid-day leaf water potential was also measured, giving a diurnal cycle. In this section the diurnal cycles of 9 May and 8 June, and the morning and evening measurements on 23 May are analysed.

9 May Figure 4.14a shows the average leaf water potential measured on 9 May. The spread between the three different plants is indicated in the plot. In the morning, ψ_l was high for both measurements, with little variation between the plants. Between the two morning measurements, there is also little variation observed in ψ_l, which indicates that there was no, or little, transpiration in between the measurements. During the morning, after 8:00, the leaf water potential decreased. At 12:30 ψ_l around -0.8 MPa were observed. The variation between the different plants at this measurement was large. One of the plants had values below -1 MPa, whereas the ψ_l for the other two plants was between -0.5 MPa and -0.7 MPa. At 13:45 , ψ_l had decreased more, with averages values of -1.25 MPa. At 18:00 a slight increase in ψ_l was observed compared to 13:30. An average ψ_l of -1.0 MPa was observed at 18:00. At 19:30, ψ_l had increased to -0.5 MPa.



(a) Average leaf water potential on 9 May, including the spread of the measurements.

(b) Average leaf water potential over height plotted for the different measurement times on 9 May.

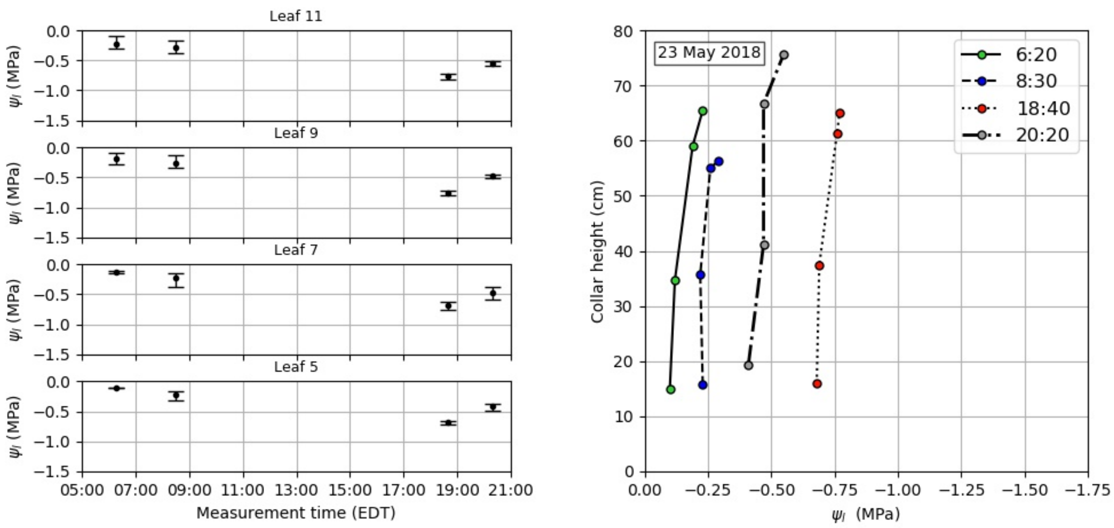
Figure 4.14: Average leaf water potential observed on 9 May.

The observations on 9 May show a clear diurnal pattern as was expected. The leaf water potential stays low in the early morning. The plant has reached an equilibrium with the soil water potential. As the transpiration increases during the day, the water losses lead to a lower leaf water potential. From the data at mid-day and in the afternoon, the lowest leaf water potential is expected to be around 15:00. As the transpiration decreases in the late-afternoon, ψ_l increases again towards an equilibrium with the soil water potential.

Figure 4.14b shows the variation in ψ_l over the height of the plant in time. In the morning the leaf water potential in the different leaves were close to each other. In the morning leaf 3 has for two out of three measurement the lowest values. This changes during the day. Then, in most measurements, the highest leaf (leaf 7) has the lowest leaf water potential. There is no uniform distribution of variation in leaf water potential over the leaves. In each measurement other leaves have the highest or second highest values. Leaf 3 had in 4 of the 6 measurements the highest variation between different plants, and leaf 7 the least variation between plants. However, later in the day, after 13:30, ψ_l for leaf 7 was lower than that of leaf 5 for all observed plants in all three measurements, see Appendix G.

23 May Figure 4.15a shows the results of the morning and evening leaf water potential measurement on 23 May. A similar pattern is observed at these times as on 9 May. As no mid-day measurements were done, the moment with the lowest plant water potential is missing. The pre-dawn ψ_l is the highest. At 8:00 ψ_l has slightly decreased. 23 May is the first day where the leaf water potential in all leaves is lower at 8:00 than at 6:00. At 19:00, ψ_l is at its lowest measured point, of approximately -0.75 MPa. This is higher than ψ_l at the same time on 9 May. The ψ_l increased again between 19:00 and 20:00 to values of around -0.5 MPa.

The leaf water potential varies only a little over height. In all measurements, the leaf water potential decreased with an increase in height, as can be seen in Figure 4.15b. For the first measurement at 6:00, leaf 5 had a leaf water potential above -0.1 MPa (-15 PSI), the exact ψ_l could not be read, as such high values were not indicated on the pressure chamber. All other leaves had equal leaf water potential of -0.1 MPa. For all measurements, the last measured plant had a lower leaf water potential, but similar trends in vertical distribution were observed in each plant.



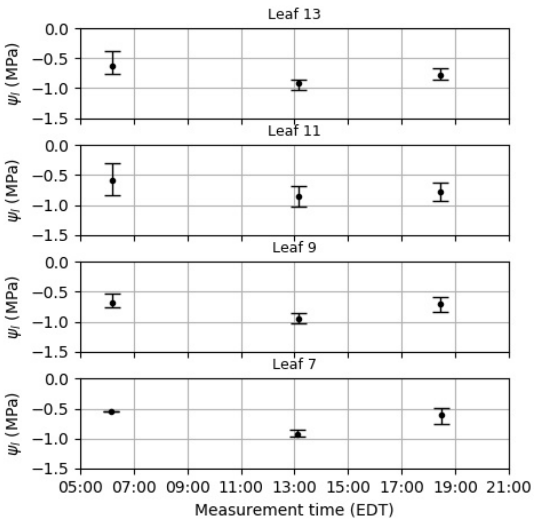
(a) Average leaf water potential on 23 May, including the spread of the measurements.

(b) Average leaf water potential over height plotted for the different measurement times on 23 May.

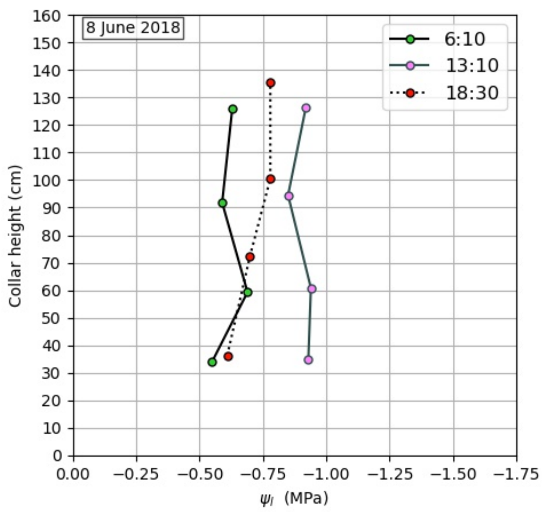
Figure 4.15: Average leaf water potential observed on 23 May.

8 June Figure 4.16a shows ψ_l on 8 June. On this day, plants were removed from the field at three times, as described in the methods. As was observed in the seasonal measurement, leaf 7 started to wilt at 6 June. The dying leaf 7 influenced the measurements on 8 June. During the morning measurements, leaf 7 was measured on only one plant. The pre-dawn ψ_l was much lower than earlier in the growing season, with values between -0.55 MPa and -0.70 MPa, compared to values around -0.25 MPa. This is caused by the low soil water potential, as described in Section 4.5.1. At 13:00, the leaf water potential had decreased until about -0.85 to -0.95 MPa. At 18:30, the leaf water potential had increased again, up to almost the initial value at 6:10 in the morning for leaf 7 and leaf 9. Leaf 11 and leaf 13 responded slower and had a lower leaf water potential for a longer time.

All leaves are very dynamic, when comparing the different plants that are observed. The spread between the different plants is high compared to the previous days, especially in the early morning. In the early morning, the leaves do not have similar ψ_l. Where in the previous days, a clear decrease in ψ_l over height was observed, this is only true for the evening measurement on 8 June. For all measurements, the spread in ψ_l is highest for leaf 11. From the 6:00 measurements, the lowest and highest ψ_l are of leaf 11. For the morning and afternoon measurement, leaf 11 has a higher leaf water potential than leaf 9. This raises the question if this is related to the ear formation and possibly also to water stress. Leaf 13 has on average a lower ψ_l than leaf 11. For the evening measurement, leaf 11 and leaf 13 have on average the same leaf water potential. In the evening, leaf 7 and leaf 9 have the highest leaf water potential. Leaf 11 is still very dynamic, but the average value is equal to that of leaf 13. Leaf 13 has the lowest leaf water potential for 2 of the 4 plants, leaf 11 has the lowest leaf water potential for the other 2 plants.



(a) Average leaf water potential on 8 June, including the spread of the measurements.



(b) Average leaf water potential over height plotted for the different measurement times on 8 June.

Figure 4.16: Average leaf water potential observed on 8 June.

4.5.3. Changes in the diurnal cycle of leaf water potential over the season

For all days, a clear diurnal cycle is observed with a high ψ_l at pre-dawn and in the early morning, a low ψ_l at midnight, and an increasing ψ_l at the end of the day, which continues after sunset. From the analysis of the diurnal measurements, it follows that ψ_l in the morning varied over the season. In this section, these observations will be further analysed. After that, the changes in the diurnal cycle over the season are analysed by dividing the growing season in the same three parts as in Section 4.4.3.

Figure 4.17 shows the difference between ψ_l, measured in at 6:00, $\psi_{l,t1}$, and the second morning measurement at approximately 8:00, $\psi_{l,t2}$. Table 4.2 shows $\psi_{l,t1}$,$\psi_{l,t2}$, the measurement times t_1, t_2, and the difference in ψ_l per day and leaf $\Delta\psi_{l,t}$, and the corresponding time difference between the two measurements Δt. A clear change in $\Delta\psi_{l,t}$ is observed. Until 23 May, $\Delta\psi_{l,t}$ is positive, meaning that ψ_l at 8:00 is higher than the pre-dawn ψ_l. This is different than the expected behaviour. As the solar radiation increases, transpiration starts and it is expected that the leaf water potential decreases, not increases as observed during this field campaign. After 23 May, the difference between the two measurements increases, $\Delta\psi_{l,t}$ decreases more further in the growing season.

There are several possible reasons for this:

- Possible errors in the early measurements or a high variation between plants
- The change in soil water potential over the season
- High influence of dew on the transpiration and the soil water availability early in the growing season
- An increasing time between the measurements over the growing season

From Figure 4.19 it can be concluded that the variation between different plants was low at both morning measurements. The same protocol in transportation and conducting the measurements was followed during the growing season. It is unlikely that measurement errors, due to the time in between measuring individual plants, would shows such a clear pattern as outcome as observed.

In the soil water potential results, it was shown that the soil water potential was high in the beginning of the season, see Figure 4.4. The decrease is soil water potential could have played some role in the difference over the season. On the 30th of May the soil water potential is high, the soil is almost saturated, and $|\Delta\psi_{l,t}|$ is also small. However, $\Delta\psi_{l,t}$ on 30 May is still below zero, while it was positive for days with lower soil water potential. On 1 June the soil water potential had the same value as in the beginning of the season, but $\Delta\psi_{l,t}$ is between -0.17 MPa and -0.27 MPa on 1 June.

During the complete growing season, heavy dew has been present, as discussed in Section 4.1.3. The dew influences the soil water content in the upper layer of the soil. Figure 4.18 shows the pattern of the soil moisture content at -5 cm and -10 cm over the period 11 May 6:00 - 15 May 6:00. During the day, a decrease in soil moisture is expected and for most of time time observed. However, between 6:00 and 10:00 a constant soil water content, or even very slight increase, is observed for each morning. This is observed through the whole season, which could be seen in less detail in Figure 4.3. The influence of the dew becomes very small and vanishes at depths of 20 cm and below.

A hypothesis is that the roots of the plant were still concentrated at low depths. Therefore, the infiltration of the dew might have had a relative high influence on the total available soil water for the plant, leading to an increase in the plant water potential. As the plants were still small, the height difference the water had to overcome was small, and it might be that it was possible for the water potential in the leaves to increase due to this small increase in soil water potential, before higher transpiration started. In case of this hypothesis, the observed trend in Figure 4.17 was the results of a combination of: (1) a relative small root system, (2) small plants and (3) the timing of the second measurement.

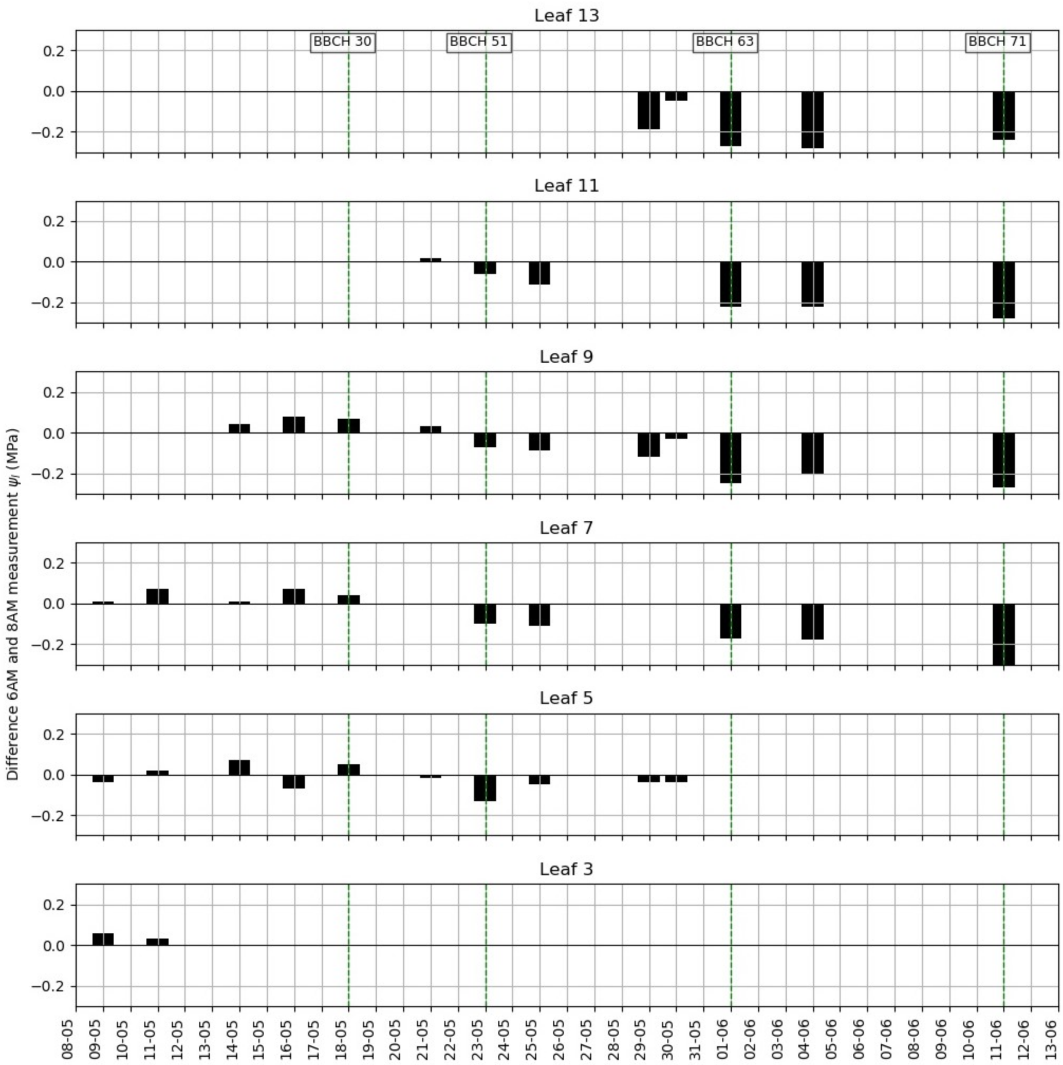


Figure 4.17: Observed differences in leaf water potential [MPa] between the morning measurement around 8:00, ($\psi_{l,2}$), and the pre-dawn measurement at 6:00, ($\psi_{l,1}$).

Table 4.2: Table with the measurement time and ψ_l of leaf 9 for the two morning measurement, and the difference between these measurements over the growing season.

Day	BBCH	h_{collar} (cm)	t_1	t_2	Δt	$\psi_{l,1}$ (MPa)	$\psi_{l,2}$ (MPa)	$\Delta\psi_l$ (MPa)
14-May-2018	19	24	06:10	08:05	1:55	-0.23	-0.19	0.04
16-May-2018	19	31	06:28	07:53	1:25	-0.29	-0.21	0.08
18-May-2018	30	41	06:17	07:53	1:36	-0.19	-0.12	0.07
21-May-2018	31	49	06:11	08:16	2:05	-0.17	-0.14	0.03
23-May-2018	32	57	06:18	08:30	2:12	-0.19	-0.26	-0.07
25-May-2018	33	66	06:18	08:12	1:54	-0.14	-0.23	-0.09
29-May-2018	55	65	06:18	08:22	2:04	-0.14	-0.26	-0.12
30-May-2018	59	66	06:21	08:10	1:49	-0.14	-0.17	-0.03
1-Jun-2018	63	61	06:24	09:16	2:52	-0.15	-0.4	-0.25
4-Jun-2018	65	63	06:32	09:22	2:50	-0.12	-0.32	-0.2
11-Jun-2018	71	69	06:14	09:02	2:48	-0.1	-0.37	-0.27

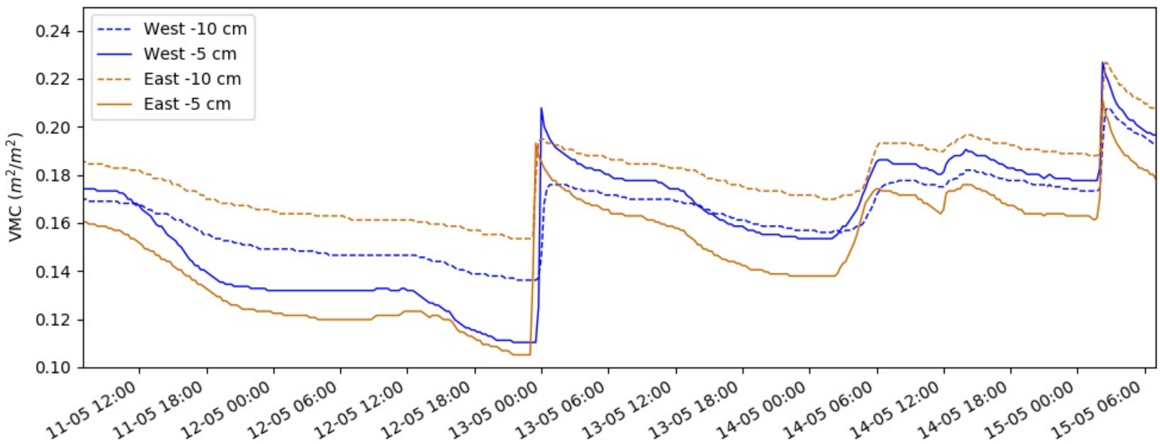


Figure 4.18: Soil moisture at 5 and 10 cm depth for the west and east pit between 11 May and 15 May.

Beginning of the vegetative stage: 26 April - 18 May (BBCH 13 - 19) In this part of the growing season, ψ_l stayed high in the morning until after 8:00. A small difference between the measurement at 6:00 and 8:00 was observed, where ψ_l at 8:00 was on most days slightly higher than ψ_l at 6:00. The variation between the different plants and between the different leaves was small. During the day, ψ_l decreased. The lowest values were reached between 14:00 and 17:00. From the data of 9 May, it follows that ψ_l at 18:30 was still low, and an increase in ψ_l took place after sunset. On 16 May, the ψ_l at 20:00 was close to the equilibrium value. This was a day with rainfall, high relative humidity, and low solar radiation in the afternoon. On 9 May, there was no cloud cover and the peak in air temperature was in the late afternoon, therefore more transpiration could take place in the afternoon, which could have lead to a smaller increase in leaf water potential. During the day, a decrease in ψ_l over height was observed. The lowest leaf had in general the highest ψ_l, and the highest leaf had the lowest ψ_l.

End of the vegetative stage: 19 May - 31 May (BBCH 30 - 59) At the start of this part of the season, ψ_l is lower at 8:00, than on 6:00. At 6:00, ψ_l stays about the same, whereas it decreases at 8:00. From 23 May onward, the leaf water potential is for all leaves lower at 8:00 than that at 6:00, as described above.

In the early morning, 6:00, the leaf water potential differences over height are slim. The difference that is observed is that leaf 5 has a little higher leaf water potential than the other leaves (except for 25 May). At 8:00 the difference between the leaves is clearly present. The lowest leaf (leaf 5) has the highest leaf water potential, and the highest leaf has the lowest leaf water potential. This is the case for all days, except the 8:30 measurement on 23 May, where the average leaf water potential of leaf 7 is 0.01 MPa higher than leaf 5. This variation over height is observed through the whole day, at 23 May. Leaf 11, the highest leaf measured that day, responds the latest to the increase in water potential at the end of the day. The leaf water potential of that leaf stays low for the longest period. The leaf water potential of leaf 5 increases the quickest.

The variation over height increases during this part of the growing season. The same pattern is observed in the period: The lowest leaf has the highest leaf water potential, and the highest leaf, the lowest leaf water potential. It is likely that the increase in variation is caused by the increased height difference between the leaves, and it might also be influenced by the lower rainfall at the end of the season. However, the soil water potential at 29 May is similar to that on the 18th, but the vertical distribution in clearer on the 29th. From this it follows that the height has a larger impact than the soil water availability.

Reproductive stage: 1 June - 11 June (BBCH 61 - 71) During this period of the growing season, the pre-dawn ψ_l decreased, this is most likely linked to the limited water availability during this period. The differences between the two morning measurements were the largest for this period of the growing season, with a difference of more than 0.2 MPa.

The lowest leaf water potential is again observed during midday, and ψ_l stays low until after sunset. In this period, senescence started. Leaf 7 started to die, on the 4th of June, and a clear difference in the leaf 7 was observed between the morning and evening. The variation over height was less clear and more dynamic than earlier in the growing season. Leaf 7 and leaf 9 were most constant and have in general a higher leaf water potential. Leaf 11 was the most dynamic, this leaf is one of the largest leaves above the ears. Leaf 13 had often the lowest leaf water potential, but it is still depended on the time, day and most of all, on the plant. In the first days, the leaf water potential decreases over height, but between 6 and 11 June, leaf 11 behaved differently: The leaf water potential at leaf 11 is higher than that in leaf 9.

There was a small variation in leaf water potential over height, when looking at the average ψ_l. On June 4th, leaf 7 was turning yellow, but not yet dying off. This was visible in the leaf water potential. At 6:00, on 4 June all leaves had nearly equal ψ_l. The difference in ψ_l over height was observed in the later measurements that day. Leaf 13 had the lowest leaf water potential, and leaf 9 the highest. In the evening, ψ_l of leaf 11 increased more between 18:00 and 20:30 than it did for leaf 13. In the evening of June 4, the variation between the measurements of one leaf increased. On the 8th of June, this variation is large for all leaves except leaf 7. On June 8, a different behaviour to the previous days was observed. All leaves behaved dynamic, there was a high variation in ψ_l at a certain leaf for the different plants. In the early morning, the leaves did not have similar ψ_l. Where in the previous days, a clear decrease in leaf water potential over height was observed, this is only true for the evening measurement on 8 June. Leaf 11 was most dynamic of all the leaves. For the morning and afternoon measurement, leaf 11 had a higher leaf water potential than leaf 9 and leaf 13. This raises the question, if this is related to the ear formation and/or by little water stress? In the morning leaf 9 had the lowest ψ_l, followed by leaf 13. During the midday measurement this was also the case, but the difference between the two leaves had deceased. In the evening, leaf 11 and leaf 13 have the lowest leaf water potential. In the evening, leaf 7 and leaf 9 have the highest leaf water potential. Leaf 11 is still very dynamic, but the average value is equal to that of leaf 13. Leaf 13 has the lowest leaf water potential for 2 of the 4 plants, leaf 11 has the lowest leaf water potential for the other 2 plants.

4.5.4. Additional results related to the leaf water potential method

Three factors related to the protocol that influence the measured ψ_l were mentioned in Chapter 2 and Section 3.7.2: (1) the height at which the sample is taken, (2) the length of the leaf sample and (3) the time difference between cutting the leaf and the measurement.

In Chapter 2, the influence of the gravitational potential on ψ_l is given. This is 0.0098 MPa/m. The assumption was made that the influence of the gravitational potential on the total ψ_l could be neglected when analysing between the different leaves. This is found to be true for these measurements. As the height variation between two measured leaves was at most 1 meter. The difference in ψ_l caused by this height difference is 0.0098 MPa, whereas the reading accuracy was 0.034 MPa.

During the fieldwork, several measurements were done to get some indication on the influence of the length of the sample on the leaf water potential. As described by Wiebe and Prosser (1977), see Chapter 2, ψ_l can vary over the length of a leaf. From these tests, it followed that the leaf water potential decreases as the sample length increases. In Appendix H the results are shown. The tests give an indication of the influence of the sample length on the leaf water potential. However, there is a large time difference between cutting the sample and the actual measurements. Moreover, re-cutting the sample can lead to errors and is therefore discouraged (Scholander et al., 1965). The length difference between the samples of the same leaf is at least 5 cm, but mostly 10 cm or more. The influence of re-cutting on ψ_l at these length differences is assumed to be less compared to re-cutting right next to the original cut, but could still be present.

One of the factors playing an important role in the accuracy of the leaf water potential measurements is the time between cutting the leaf and taking the measurement, see Section 3.7.2. This was a challenge for the leaf water potential measurements, as the pressure chamber was located at 5 minutes from the field. In order to limit the influence of time difference, during the first protocol, on 4 and 7 May, the plants were measured one by one. Before each measurement, new plant had to be transported from the field to the garage, as described in the methods in Section 3.7.2. Because of this, only the first measurement was then taken at pre-dawn. The first three measured plants were used as 6:00 values and the last three measured plants as 8:00 values.

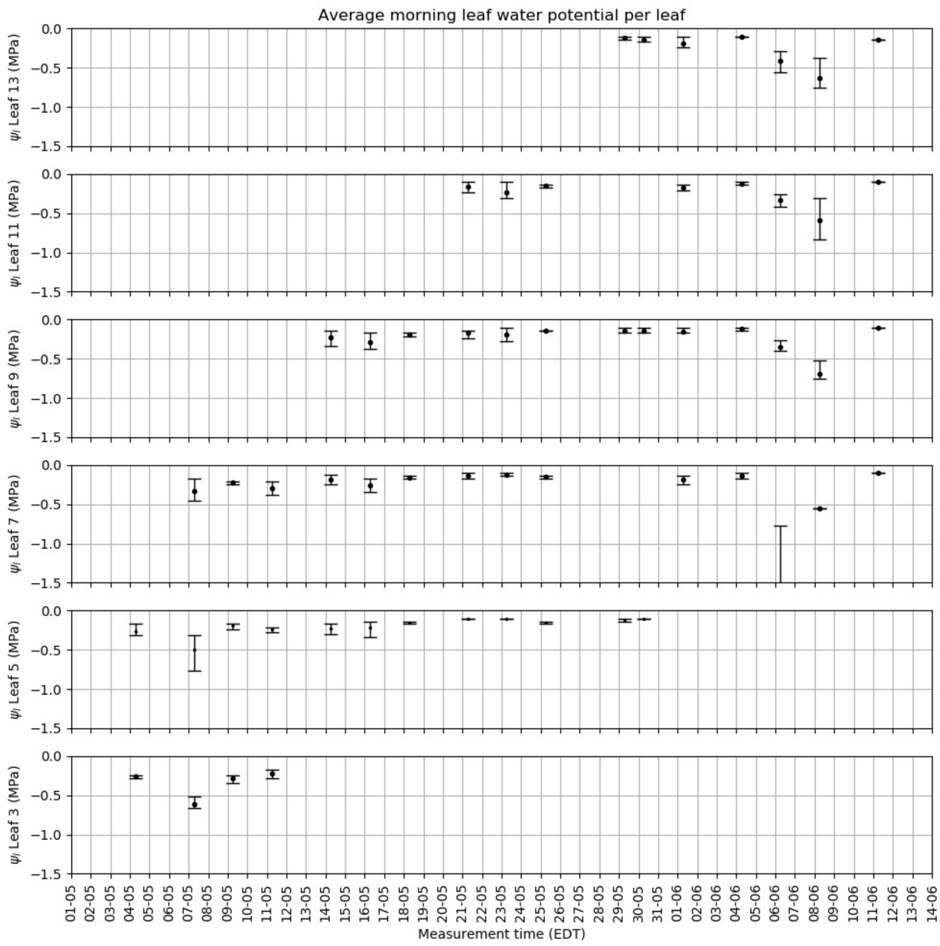


Figure 4.19: Spread of each leaf at the 6AM morning measurements.

Figure 4.19 shows the spread in leaf water potential of three measured plants at 6:00 for each leaf number. It can be seen that the spread for each leaf number, and the variation between the different leaves, are larger for the measurements done in the old protocol. These differences between the measurements are a result of both the variation between plants, and the influence of cutting time. Since the plants are cut at different times, it is hard to tell what the impact of the time difference is. As the plants grew taller the time between cutting the leaf and doing the actual measurement took longer on 7 May, and was expected to increase over the growing season. This observation lead to the use of the second protocol as described in the methods, Chapter 3, Section 3.7.2 The data of 4 May and 7 May were excluded from further analysis.

In the second protocol, the field removal time was equal for the different plants. The spread in the leaf water potential measured between the plants is mostly determined by the differences between the different plants and not by transpiration of the plants in the garage. On some days, a decrease in leaf water potential of the measured plants in time was observed. This was caused by the waiting time in the garage. These differences were only observed on 11 May, 25 May, 1 June, 6 June and 8 June, and less than the difference observed for 4 and 7 May.

4.6. Sap flow

Sap flow measurements were conducted from 18 May 2018, until the harvest. The complete sap flow data analysis can be found in Vermunt et al. (2019). In this report, the sap flow data for 11 June will be analysed in combination with the solar radiation and stomatal conductance, see Figure 4.20. More results from diurnal sap flow measurements are shown in Appendix I.

As can been seen in Figure 4.20, the pattern in solar radiation is also observed in the sap flow, but with a delay. This is the case for both plants. The sap flow measured in the plant on the East side is for the whole season less than that in the plant on the West side. The sap flow is decreasing 15-30 minutes after the solar radiation decreased. The increase in sap flow is observed to be 30-45 minutes after the increase in solar radiation. The sap flow shows a similar pattern as the stomatal conductance, see Section 4.4, but with a larger time difference to the solar radiation. In the evening, the stomata in top leaves were open until 20:00, although the solar radiation was already decreasing. After the stomata closed, sap flow still continued until 21:00, increasing the water content in the plant.

At around 8:45 in the morning, the sap flow rapidly increased. This can be related to the dew formation on the leaves. Transpiration is determined by the opening of the stomata in combination with the VPD. When dew is present on the leaf, the VPD is close to zero, and the transpiration is therefore low (Ben-Asher et al., 2010). When in the morning the transpiration of the plant is low, this will result in a low sap flow. The sensor can be less sensitive to low flows; these flows might not be registered. The attachment of the sensor influences this sensitivity. When the transpiration and thus the sap flow increases, the sensor is able to detect the flow. On other days, the East sensor shows a more graduate increase in sap flow in the early morning, before it is detected by the West sensor, see Appendix I.

The sap flow measurement is interesting to do in combination with the stomatal conductance measurements. It provides continues data, and it is likely that the difference in stomata response to water stress is visible in the sap flow data. If this is the case, the sap flow data can be used to determine the most interesting moments for the stomatal conductance measurements. However, it should be taken into account that the sap flow sensors can be installed only when the stem diameter is at least 15 mm. The sap flow can therefore not be measured early in the growing season.

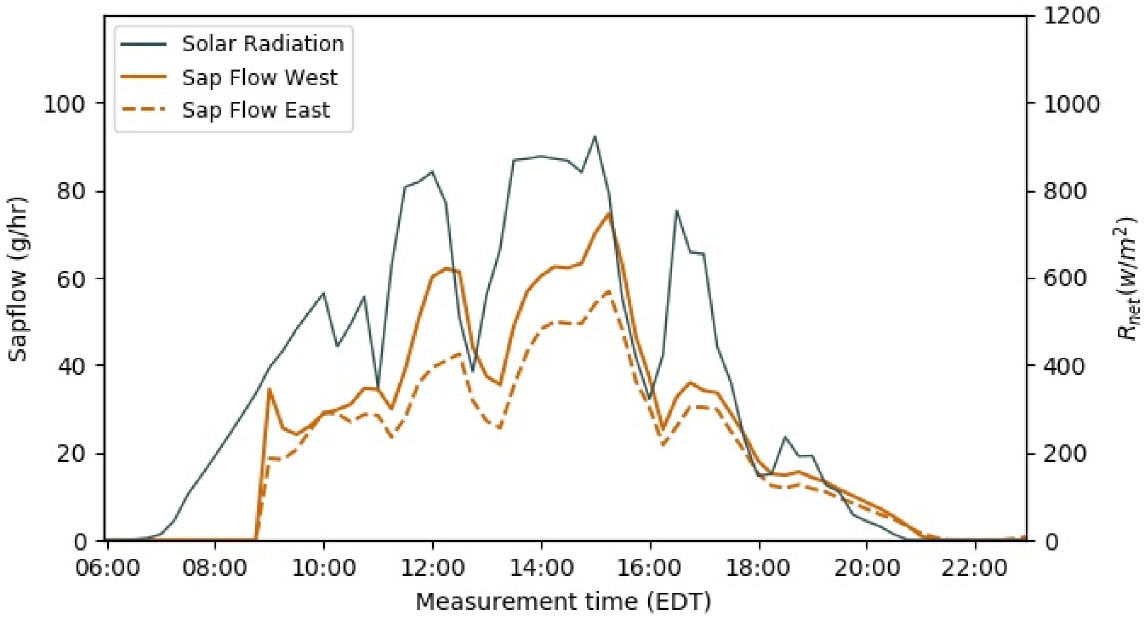


Figure 4.20: Sap flow data and solar radiation for 11 June. Data derived from Vermunt et al. (2019).

4.7. Synthesis

In the stomatal conductance g_s, a large variation over height is observed. The shaded leaves have a low g_s, whereas the higher leaves that are more exposed to solar radiation have a high g_s. This difference over height in g_s is not observed so clearly in the ψ_l. During the season, a small variation over height was observed when considering the averages of the three measured plants, where ψ_l decreased over height. This small variation can be observed in Figure 4.13, and in the diurnal Figures. In Appendix G the exact difference between the measured leaves is given. However, only for few measurements times this variation was observed for all three measured plants. The variation between the leaves seems to increase as the plant has less water available. From these observations, it can be expected that, as a result of stomatal closure, the difference in ψ_l over height increases over height as the plant has less water available. Therefore, the measurements might be of more interest for water stressed corn than for well-watered corn.

The only day on which multiple measurements were conducted for both ψ_l and g_s was 23 May. On this day, g_s was low for leaf 5 during the whole day. The stomatal conductance increased over height, as the leaves became more exposed to the solar radiation. Leaf 9 and leaf 11 have similar g_s values. Over the complete day, ψ_l shows a small variation over height. However, when looking at the spread of the measurements, this difference in height can be contributed to the variation between the measured plants, rather than a significant difference in ψ_l over height in general. Between the leaf water potential measurements of 18:40 and 20:20, a fast increase is observed. Although the stomatal closure has not been measured on 23 May, when assuming a behaviour similar to the other days, the stomata were likely to close between 19:00 and 20:00. The closure in stomata caused a rapidly decreased of the transpiration and allowed for the plant water potential to increase. This is observed in the difference in ψ_l between 18:40 and 20:20.

As described in Chapter 2, plants are divided into two groups: isohydric and anisohydric plants. Corn is identified as an isohydric species (Tardieu and Simonneau, 1998), which means that the stomatal behaviour should change to maintain the midday ψ_l. From literature, it is expected that when corn is in the reproductive stage (flowering, BBCH 60-69) the stomata change in behaviour when ψ_l decreases to a value below -1.7 MPa (see Chapter 2). In the results no clear difference in g_s as a result of limited leaf water potential has been observed. This was also expected since no leaf water potential values lower than -1.3 MPa have been observed. This is higher than the critical value observed in earlier research (Tardieu and Simonneau, 1998; Turner, 1974). The value of -1.3 MPa was observed early in the growing season on 9 May (BBCH 17), the lowest leaf water potential measured after that was around -0.95 MPa on 8 June (BBCH 65). From this measurements, it can be concluded that ψ_l had not reached the value for which a clear relation between ψ_l and g_s can be observed, therefore it is difficult to draw conclusions on the link between them.

However, a clear response in ψ_l to low ψ_s was observed on several days late in the growing season. From this we can conclude that for well-watered crops, ψ_l can decrease, but the stomata seem to keep behaving in a similar way as long as ψ_l does not decrease below a critical value of -1.7 MPa. At least on the days where ψ_l was measured for the full day. On 6 and 8 June a lower ψ_l was observed at pre-dawn, with the lowest values at 8 June. Unfortunately, due to time constrains it was not possible to measure g_s on this day. Therefore, no indication can be given on any possible change in g_s. From the sap flow results in Section 4.6 it followed that the sap flow data can give insight in the stomatal behaviour and g_s. For 8 June, a low sap flow in the afternoon is observed. However, as there is limited solar radiation for most of the afternoon, no conclusions can be drawn regarding early stomatal closure due to limited water availability.

By comparing the sap flow data with the morning observations in ψ_l, a better understanding of the observations can be obtained. In Section 4.5.3 a high difference between the two morning observations was observed in the end of the season. Table 4.2 provides information on the measurement times. When this data is combined with the sap flow data, an rapid increase in sap flow is observed between 8:30 and 9:00. This corresponds with the measurement times and observations from Section 4.5.3. On the days where the second measurement was done after 9:00 a large difference between the two morning measurements was observed. However, it does not explain all difference, as was also discussed in the section. The sap flow measurements supports conclusions drawn in Section 4.5.3 regarding the influence of the measurement times.

5

Recommended Protocol

In this research, it was aimed to characterize the variation in stomatal conductance and leaf water potential for the use of radar experiments. For future field experiments with a similar aim, several changes in the protocol are recommended. This includes recommendations on the design and set-up of the field experiments, and recommendations regarding the actual measurements of the stomatal conductance and leaf water potential. In this chapter, the currently used protocols will be discussed and recommendations will be given. With these recommendations, the possibility of comparing water stressed crops with well-watered crops is taken into account.

5.1. Design of fieldwork

A general recommendation regarding the design of the fieldwork is to pick the right season. As the stomatal conductance measurements cannot take place on wet leaves, a season with limited rainy days is preferred. If measurements will be done at the same location, this would mean planting the corn in March. In this way, most measurements can be done before mid-May, when generally the summer afternoon rains start. During the field campaign, the afternoon rains made stomatal conductance measurements in the afternoons impossible. Preventing this in future fieldwork is especially important when water stress would be imposed on part of the field. Early stomatal closure is expected to be observed in the afternoon for water stressed crops.

The combination of data on the stomatal conductance and leaf water potential for the same days gives information on water stress and response of the plant at different heights. It is recommended to measure two full days, from dawn until sunset, instead of one full day and two days from morning until early afternoon. To capture changes in the diurnal cycle of the stomatal conductance, it is necessary to measure until sunset. Measurements until mid-afternoon give an indication of the diurnal cycle, but do not allow hard conclusions.

To be able to compare the stomatal conductance measurements with the leaf water potential measurements, it is desired to have measurements at approximately the same time and at the same leaves. This is especially important for crops in water-stressed conditions. In a well-watered canopy, the variation in stomatal conductance is well captured in this study. The results show that the variation in stomatal conductance is determined by the solar radiation, and the variation over height therefore by the shading of the leaves. For the leaf water potential, some variation was observed over height, but this was small. Measuring only leaf 11 could be sufficient to analyse the plant water dynamics for a well-watered canopy. For a water-stressed canopy, measurements over different heights of the plant are strongly encouraged.

From this research and previous studies followed, that the leaves around the ear and the leaves just above the ear will be of most interest, as there will likely be a difference in response to water stress in these areas (Steele-Dunne et al., 2016; Van Emmerik et al., 2017). These leaves have a high water content, and are exposed to solar radiation. From the stomatal conductance measurements, it followed that leaf 11 had the highest maximal stomatal conductance, and the highest variation. Optimally, the leaves around the ear, plus one leaf above the ear are measured. If there is limited time and resources available, the recommendation is to measure the leaf above the ear for a full grown canopy. In this research that would have been leaf 11. This leaf showed a dynamic response in leaf water potential when there was a low soil water potential, besides that leaf 11 had a high exposure to solar radiation and it is therefore likely that early stomatal closure due to water stress can be observed in this leaf.

Combining the sap flow observations with both the leaf water potential and the stomatal conductance results, proved to give useful insights. The sap flow measured continuously, something that is not possible for either leaf water potential or stomatal conductance measurements. If is therefore recommended to include sap flow measurements if possible. To be able to obtain a more complete understanding of the soil water availability of the plant, it is recommended to determine the root zone depth of the corn plants. This can be done once a week by digging out a plant.

5.2. Leaf water potential measurements

Measurement times The main recommendation regarding the leaf water potential measurements is to include weekly measurements at 14:00. At this time of the day, the plants experience most water stress, as the transpiration is highest and the ψ_l is lowest. The pre-dawn (6:00) and mid-day (12:00) leaf water potential give most insight in the water status of the plant.

Measurement location Most challenges of the leaf water potential measurements were related to the location of the pressure chamber. The chamber was located in a garage about 5 minutes' driving from the field, which resulted in the protocol as described in Section 3.7. There are several disadvantages to this method. The atmosphere and the light in the garage is different to the outside atmosphere and light. This influences the transpiration of the plants. Although this effect was found to be limited, it is preferred to conduct the measurements in atmospheric conditions similar to the field. Another disadvantage was the damage of sample leaves due to transportation. The most important disadvantage was the difficulty of finding three undamaged plants that were representative for the field. Late in the season this took up to 45 minutes on certain days, leading to an increasing time difference between the measurements.

As both sides of the field got the same amount of irrigation and precipitation, the location of the pressure chamber was inconvenient, but did not cause problems for conducting this research. However, in future research, a comparison between fields with different water availability might be desired. To be able to compare the results from both situations, limited time difference between the measurements is desired. This is not possible in the current situation.

To obtain the limited time difference between the measurements, and to eliminate the challenges described above, it is recommended to locate the pressure chamber in, or close to, the field. This makes it possible to cut the sample leaves of one plant at the same time, measure them and collect the sample leaves of the next plant after that, instead of collecting all three plants at the same time. Doing so has the following advantages:

- No root damage of the sample plants;
- Limited in transpiration because of a change in light source and surrounding atmosphere;
- No damage of plants during the transportation to the pressure chamber;
- Being able to cut the leaves of the plants in the field;
- Easier to select plants, as there is no need to have three adjacent plants without damages;
- Less time pressure on conducting the measurements;

When placing the pressure chamber in or close to the field, a safety analysis should be done before executing the measurements.

Measured plants and leaves It is recommended to measure 3 plants for each situation, water stressed or well watered. When less samples are taken, the heterogeneity of the field is not taken into account. Especially in the situation of water stress, the variation between plants is expected to increase. From the result of this study, a small, not consistent decrease in ψ_l over height was observed. This is assumed to increase when the plant experiences water stress. For water-stressed conditions, it is therefore of importance to measure the leaf water potential at different heights. The same leaf numbers should be measured for the leaf water potential and the stomatal conductance. Although measuring at different heights for well-watered corn would give a good comparison, time can be a limiting factor. In that case, measuring for well-watered conditions only the leaf above the ear (leaf 11 in this case) might give sufficient insight regarding the leaf water potential.

Sample length At the current fieldwork, the sample length in proportion to the full leaf length varied. In this analysis, the possible errors of this variation is not taken into account. The plants were well watered and the samples cut at the maximal possible length to make it fit in the pressure chamber. However, from the additional measurements and literature, it followed that the sample length does influence the leaf water potential. This is especially the case for water stressed crops. Therefore, it is recommended to include the sample length as ratio of the total leaf length in the protocol. From the executed field experiments, cutting the samples at 2/3 of the total leaf length from the tip is reasonable. The influence of water stress will be observed, but the leaf water potential is likely to increase again as the plant water potential increases (Wiebe and Prosser, 1977). More research on the influence of the leaf length on the measured leaf water potential is recommended.

General recommendations regarding the leaf water potential measurements:

- Place a damp paper towel in the plastic bag with the samples before measuring to prevent transpiration from taking place. This is not needed when the plastic bag fits closely around the leave and can be placed in the pressure chamber with the leaf.
- If the plastic bag is too wide, it cannot be places in the pressure chamber with the leaf. The plastic gets sucked out of the chamber when it empties, preventing good exhaustion of the chamber. Therefore, in case of loose plastic bags, place a damp paper towel in the pressure chamber before the measurements and remove the leaf from the plastic when doing the measurements.
- The gasket should not be closed too tight, as the nerve of the leaf will break.
- If the chamber lid does not close easily, lubricating the outside of the o-ring helps.
- Make sure the screws in the chamber lid are tight enough to prevent the gasket from moving. Not attaching the screws tight enough can result in a leak after several measurements.
- Start the measurements with the valve at a low rate. If the plants are not well-watered, one can slowly open the valve more. It is recommended to decrease the rate when the pressure is close to the end point, to increase the accuracy of the measurement.

5.3. Stomatal conductance measurements

Measurement time It is recommended to start the measurements not earlier than BBCH stage 15. During the measurement on 26 April (BBCH 13), the leaves were very fragile. When removing the porometer, the leaves were easily torn. Also, the plants had a low biomass and a LAI of 0.04. Therefore, the influence of the corn plants on the back-scatter detected by the radar will be limited. The need for characterizing the stomatal behavior before BBCH stage 15 is limited.

The timing of the diurnal measurements should be focused on capturing the stomatal closure. The stomatal conductance can be captured by measuring every three hours from dawn until sunset. It is recommended to be, if possible, more precise with the starting time of the measurements.

Measured plants and leaves Stomatal conductance measurements are preferably done on three plants for each field condition. The plants are preferably close to each other, with at least 4 plants in between. This will limit the time difference between measurements, but still give the possibility to use neighbouring plants in case of damage during the growing season. Stomata have a quick response, especially to a difference in radiation. To be able to make a comparison between the measurements, the time difference must be as short as possible. This is also the case for a comparison between well-watered and water stressed plants. If the field is set-up to have half the of the field well-watered and the other half with reduced irrigation, choosing plants in the same row and close to the middle of the field, limits the walking time between the measurements.

The leaves around the ear and in the top of the canopy are the most interesting for two reasons: (1) The stomatal conductance of the higher leaves is not restricted by shade from other leaves. Early stomatal closure is therefore best observed in these leaves. (2) The leaves around the ear and just above the ear contain most water. As mentioned before, water influences the backscatter that is sensed with radar measurement. If there is a difference in water dynamics in these leaves in a water stressed situation compared to a well watered situation, this might be detected by the radar.

General recommendations regarding the stomatal conductance measurements:

- The Leaf Porometer should be calibrated each day before the measurements are taken. Ensure that the sensor is in thermal equilibrium with the environment in the field before calibration.
- Measure the collar height of the leaves during the first measurement of the day. This makes it possible to compare the results well with the results of other measurements, and prevents confusion of leaves during the season, as the collar heights change less than the maximum leaf height.
- Measure the height at which the measurement is taken during the first measurement of the day. The temperature and relative humidity changes within the canopy over height. As the measurement height on leaves with similar collar height can differ a lot, knowing the height of the taken measurement might help to understand possible difference in measured stomatal conductance.

6

Conclusions and Recommendations

This study aims to characterize the variation in stomatal conductance and leaf water potential of corn plant in height and time through the growing season under water stressed and well-watered conditions. This aim was researched by answering four research questions. Unfortunately, due to unforeseen circumstances, it was not possible to impose water stress on the corn plants. Therefore, the study has only been conducted for well-watered corn. However, the research questions could be answered for the well-watered conditions. A conclusion for each of the questions is given below, followed by a final conclusion and recommendations.

What is the variation of the stomatal conductance and leaf water potential over the height of the plant?
The stomatal conductance is highly dependent on light. The leaves that receive a more solar radiation have a higher stomatal conductance. When all leaves are small and able to receive full solar radiation, the stomatal conductance is similar for the different leaf numbers. When the crops grow and the lower leaves become more shaded, a difference in stomatal conductance over height is observed. The canopy can be described as different layers, which develop during the growing of the crop: A layer of leaves fully exposed to the solar radiation, with hardly any shading by other leaves (layer 1), a layer with leaves that are both shaded and sunny, the shading of the leaves varies per plant and time of the day (layer 2), and lastly the lowest layer with fully shaded leaves (layer 3). The lower, shaded leaves (layer 3) have a low stomatal conductance. The leaves in layer 2 have a higher stomatal conductance than the completely shaded leaves of layer 3, but less than the leaves that are fully exposed to the solar radiation (layer 1). The stomatal conductance for these leaves is high, and the variation between the different measurements is also high. Early stomatal closure caused by limitations such as water stress can be observed in Layer 1.

The leaf water potential shows a small decrease with the height of the plant. The difference over height is most related to the soil water availability and transpiration, with low soil water availability and high transpiration the difference over height increased. Also, when the plant is small, this difference is limited, but when the height of the plant increases, the difference is more distinct. In the last stages (BBCH 65+), the variation over height is changing. The lower leaf (leaf 9) had a more stable, and on average higher leaf water potential. The highest measured leaf, leaf 13, had the lowest leaf water potential. But the middle leaf, leaf 11, had a dynamic behaviour. As lower leaves start to senesce, the leaf water potential in these leaves decreases to very low values compared to the measured values in the other leaves.

What is the diurnal cycle of the stomatal conductance and the leaf water potential of maize, in water stressed and non-water-stressed plants?
The stomatal conductance could only be measured after 10:00, when the dew had disappeared. The stomatal conductance was high, but still increasing by that time. The maximum stomatal conductance was measured around 14:00. In the beginning of the growing season the moment of maximum stomatal conductance was slightly later, and towards the end the moment of maximum stomatal conductance was earlier. The stomatal conductance decreased rapidly with decrease of the solar radiation. At the end of the day, the stomata close rapidly. At the end of the season, on June 8th, the stomata closure starts earlier in the day. This can be caused by water shortage.

For the leaf water potential, a similar diurnal cycle is observed as is described in literature. At 6:00 in the morning, all leaves have a similar leaf water potential, which is depending on the soil water potential. The leaf water potential decreases and reaches a minimum in the afternoon, between 14:00 and 17:00. In most cases, the highest leaf has the lowest leaf water potential, with a minimal -1.3 MPa on 9 May. At the end of the day, the leaf water potential started to increase again. Depending on the water availability in the soil, leaf water potential values close to the early morning values could already be reached in the evening. In the leaf water potential, a difference is observed between days with a high soil water potential and those with a low soil water potential. If the soil water potential was high, a smaller decrease in leaf water potential was observed at the end of the day.

How do the diurnal cycles in stomatal conductance and leaf water potential change over the growing season?

For the change in diurnal cycle for stomatal conductance, only the sunny leaves are considered. In the beginning of the season, the maximal stomatal conductance is around $800 mmol/m^2s$, there is no clear difference in the measured stomatal conductance at 11:00 and 15:00. After 15:00, the stomatal conductance starts to decrease. In the middle on the growing season (BBCH 30-59) the stomatal conductance keeps increasing until around or just after midday, 12:00-14:00. Values between $1000 - 1200 mmol/m^2s$ were observed for sunny leaves. The stomatal conductance stays high until the sunsets. In the last part of the growing season, where the ears start to develop, no measurements on a fully sunny day where done. The sparse amount of data limited a reliable analysis on the change in diurnal cycles for the last part of the growing season. On the last day, 11 June, the diurnal cycle could still be observed. Here a decrease in stomatal conductance was observed from 14:00 onward. This could be linked to the growing stage or to the limited water availability on this day, as other environmental conditions were comparable to days with diurnal measurements earlier in the season.

The leaf water potential at 8:00 decreases over the growing season. The difference between 6:00 and 8:00 measurements becomes larger, and more negative. This was caused by a combination of measuring time, but not only. Other reasons that might have influences this increasing difference in morning leaf water potentials are expected to be related to the root zone depth and the height of the plant.

How do the changes in the stomatal conductance relate to changes in the leaf water potential and the other way around?

The water vapor loss through the open stomata cause a decrease in the leaf water potential. In the relation between leaf water potential and stomatal conductance, the distinction can be made between isohydric and anisohedric species. The isohydric species have an early stomatal closure in case of water stress, to prevent a decrease of the leaf water potential. From the collected data, this behavior is not clearly observed, mainly because there was sufficient water available during all measurements. Although there have been observations with a lower leaf water potential than other days, it is most likely that the leaf water potential has not yet reached the threshold value of the leaf water potential for which early stomatal closure takes place. The only day where the leaf water potential could have reached this value is on June 8. To understand the relation between stomatal conductance and leaf water potential in more detail, more research on stomatal conductance and leaf water potential for corn in water stressed conditions is needed, including measurements for both the stomatal conductance and leaf water potential in the middle of the day, on the same day.

Various recommendations can be given after conducting this research. The most important, and obvious recommendation is to conduct leaf water potential and stomatal conductance measurements over the growing season under water-stressed conditions. From the limited obtained data on canopy with lower soil water potential, it is expected that variations over height will be observed in the leaf water potential for water stressed crops. From study by Van Emmerik et al. (2017) showed a variation in backscatter well-watered and water-stressed crop, which is most likely influenced by the water content in the crops. To be able to link the observations in leaf water potential to radar, the water content in the corn should be measured at the same times as the leaf water potential. The leaf water potential is an indicator for stress, but the water content is measured by radar. In the current measurements, a complete diurnal cycle was only measured on 2 days. On the other days, part of this cycle was observed, which gave valuable insights. However, for future measurements, fewer complete diurnal measurements are recommended over more days with measurements, but where no complete cycle was measured. For the pre-dawn measurements, keeping a high measurement frequency is recommended, as this is an important indicator for water stress. The observed results can be used as a guideline for timing of the diurnal cycles. During each measurement, the plants were measured at three or four heights. Considering the duration of the measurements, the low variation over height in leaf water potential, and the obvious variation in height for the stomatal conductance, measuring only at one height can be sufficient to capture the diurnal dynamics for well-watered corn. From this research, it is recommended to measure the a leaf that is exposed to solar radiation, but not completely in the top of the plant. For fully grown corn, this could be the fourth leaf from the top, or leaf 11 for the corn in this study. Last, it is highly recommended to include sap flow measurements when observing the leaf water potential and stomatal conductance. The sap flow is driven by the transpiration and shows similar patterns over time as the stomatal conductance measurements. The largest benefit of the sap flow data is that it is continues data. This provides insight in the time of the day where important changes in plant-water dynamics take place.

Bibliography

Allen, R. G., Luis, S. P., RAES, D., and Smith, M. (1998). FAO Irrigation and Drainage Paper No. 56. Crop Evapotranspiration (guidelines for computing crop water requirements). *Irrigation and Drainage*, 300(56):300.

Araújo, W. L., Fernie, A. R., and Nunes-Nesi, A. (2011). Control of stomatal aperture: A renaissance of the old guard. *Plant Signaling and Behavior*, 6(9):1305–1311.

Assmann, S. M. and Shimazaki, K.-i. (1999). Update on Signal Transduction The Multisensory Guard Cell . Stomatal Responses to Blue Light and Abscisic Acid 1. *Plant Physiology*, 119(March):809–815.

Baret, F., Madec, S., Irfan, K., Lopez, J., Comar, A., Hemmerlé, M., Dutartre, D., Praud, S., and Tixier, M. H. (2018). Leaf-rolling in maize crops: From leaf scoring to canopy-level measurements for phenotyping. *Journal of Experimental Botany*, 69(10):2705–2716.

Ben-Asher, J., Alpert, P., and Ben-Zvi, A. (2010). Dew is a major factor affecting vegetation water use efficiency rather than a source of water in the eastern Mediterranean area. *Water Resources Research*, 46(January):1–8.

Bongiovanni, T., Liu, P.-w., Nagarajan, K., Preston, D., Rush, P., Duan, X., Chen, G., Terwilleger, R., Monsivais-huertero, A., Judge, J., De Roo, R., Akbar, R., Morris, M., Williams, O., Marks, L., Cardozo, C., Moghaddam, M., and England, A. (2015a). Field Observations during the Tenth Microwave Water and Energy Balance Experiment (MicroWEX-10): from March 1, 2011, through January 5, 2012. Technical report, UF/IFAS, Gainesville.

Bongiovanni, T., Liu, P.-W., Nagarajan, K., Preston, D., Rush, P., van Emmerik, T. H., Terwilleger, R., Monsivais-Huertero, A., Judge, J., Steele-Dunne, S. C., De Roo, R., Akbar, R., Baar, E., Wallace, M., and England, A. (2015b). Field Observations During the Eleventh Microwave Water and Energy Balance Experiment (MicroWEX-11): from April 25, 2012, through December 6, 2012. Technical report, UF/IFAS, Gainesville.

Boyer, J. S. (1970). Differing sensitivity of photosynthesis to low leaf water potentials in corn and soybean. *Plant Physiology*, 46(2):236–239.

Cakir, R. (2004). Effect of water stress at different development stages on vegetative and reproductive growth of corn Recep. *Field Crops Research*, 89:1–16.

Cozzolino, D. (2017). The role of near-infrared sensors to measure water relationships in crops and plants. *Applied Spectroscopy Reviews*, 52(10):837–849.

Damm, A., Paul-Limoges, E., Haghighi, E., Simmer, C., Morsdorf, F., Schneider, F. D., van der Tol, C., Migliavacca, M., and Rascher, U. (2018). Remote sensing of plant-water relations: An overview and future perspectives. *Journal of Plant Physiology*, 227(April):3–19.

Donovan, L. A., Richards, J. H., and Linton, M. J. (2003). Magnitude and Mechanisms of Disequilibrium between Predawn Plant and Soil Water Potentials. *Ecology*, 84(2):463–470.

Driscoll, S. P., Prins, A., Olmos, E., Kunert, K. J., and Foyer, C. H. (2006). Specification of adaxial and abaxial stomata, epidermal structure and photosynthesis to CO2enrichment in maize leaves. *Journal of Experimental Botany*, 57(2 SPEC. ISS.):381–390.

Dwyer, L. M. and Stewart, D. W. (1984). Indicators of Water Stress in Corn (Zea mays L.). *Canadian Journal of Plant Science*, 64:537–546.

Earth Observation and Research Branch Team (2011). Crop Identification and BBCH Staging Manual: SMAP-12 Field Campaign. Technical report.

Jarvis, P. G. (1986). Stomatal Control of Transpiration: Scaling Up from Leaf to Region. *Advances in Ecological Research*, 15:1–49.

Joerg, H., Pardini, M., Hajnsek, I., and Papathanassiou, K. P. (2018). Sensitivity of SAR Tomography to the Phenological Cycle of Agricultural Crops at X- , C- , and L-band. *IEEE Journal of Selected Topics in Applied Earth Observations and Remote Sensing*, 11(9):3014–3029.

Kangur, O., Kupper, P., and Sellin, A. (2017). Predawn disequilibrium between soil and plant water potentials in light of climate trends predicted for northern Europe. *Regional Environmental Change*, 17(7):2159–2168.

Kim, T.-H., Böhmer, M., Hu, H., Nishimura, N., and Schroeder, J. I. (2010). Guard Cell Signal Transduction Network: Advances in Understanding Abscisic Acid, CO 2 , and Ca 2+ Signaling. *Annual Review of Plant Biology*, 61(1):561–591.

Lambers, H., Stuart Chapin III, F., and Pons, T. L. (2008). *Plant Physiological Ecology*. Springer Science+Business Media, LLC, New York, second edi edition.

Ma, B., Pu, R., Zhang, S., and Wu, L. (2018). Spectral Identification of Stress Types for Maize Seedlings Under Single and Combined Stresses. *IEEE Access*, 6:13773–13782.

Mi, N., Cai, F., Zhang, Y., Ji, R., Zhang, S., and Wang, Y. (2018). Differential responses of maize yield to drought at vegetative and reproductive stagess. *Plant, Soil and Environment*, 64(6):260–267.

Neumann, H. H., Thurtell, W., and Stevenson, K. R. (1974). In situ measurements of leaf water potential and resistance to water flow in corn, soybean, and sunflower at several transpiration rates. *Canadian Journal of Plant Science*, 54:175–184.

Nobel, P. S. (2009). *Physicochemical and Environmental Plant Physiology*. Elsevier, 4th edition.

Peel, M. C., Finlayson, B. L., and McMahon, T. A. (2007). Updated world map of the Köppen-Geiger climate classification. *Hydrology and Earth System Sciences*, 11(5):1633–1644.

Petruzzellis, F., Savi, T., Bertuzzi, S., Montagner, A., Tretiach, M., and Nardini, A. (2018). Relationships between water status and photosystem functionality in a chlorolichen and its isolated photobiont. *Planta*, 247(3):705–714.

Programme)/UN-Water, W. U. N. W. W. A. (2018). The United Nations World Water Development Report 2018: Nature-Based Solutions for Water. Technical report, UNESCO, Paris.

Raschke, K. (1970). Stomatal Responses to Pressure Changes and Interruptions in the Water Supply of Detached Leaves of Zea mays L. *Plant Physiol*, 45:415–423.

Ray, P. M. (1972). *The Living Plant*. Holt, Rinehart and Winston, Inc., New York, second edi edition.

Reicosky, D. C. and Lambert, J. R. (1978). Field measured and simulated corn leaf water potential. *Soil Science Society of America Journal*, 42(2):221–228.

Riboldi, L. B., Oliveira, R. F., and Angelocci, L. R. (2016). Leaf turgor pressure in maize plants under water stress. *Australian Journal of Crop Science*, 10(6):878–886.

Sade, N., Gebremedhin, A., and Moshelion, M. (2012). Risk-taking plants. *Plant Signaling and Behavior*, 7(7):767–770.

Scholander, P. F., Hammel, H. T., Bradstreet, E. D., and Hemmingsen, E. A. (1965). Sap Pressure in Vascular Plants. *Science*, 148:339–346.

Seminis (2015). Sweet Corn Growth Stages and GDUs. Technical report, Seminis Vegetable Seeds, St. Louis, Missouri.

Shimazaki, K.-i., Doi, M., Assmann, S. M., and Kinoshita, T. (2007). Light Regulation of Stomatal Movement. *Annual Review of Plant Biology*, 58(1):219–247.

Slavik, B. (1963). The Distribution Pattern of Transpiration Rate , Water Saturation Deficit , Stomata Number and Size , Photosynthetic and Respiration Rate in the Area of the Tobacco Leaf Blade In the study of distribution patterns of osmotic pressure of cell-sap on the Tr. *Biologia Plantarum*, 5(2):143–153.

Steele-Dunne, S. C., Friesen, J., and Van De Giesen, N. (2012). Using diurnal variation in backscatter to detect vegetation water stress. *IEEE Transactions on Geoscience and Remote Sensing*, 50(7 PART1):2618–2629.

Steele-Dunne, S. C., McNairn, H., Monsivais-Huertero, A., Judge, J., Liu, P.-W., and Papathanassiou, K. (2016). Radar Remote Sensing of Agricultural Canopies: A Review. *Remote Sensing of Environment*, 187(6):1607–1621.

Tardieu, F. and Simonneau, T. (1998). Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. *Journal of Experimental Botany*, 49(Special):419–432.

Turner, N. C. (1973). Stomatal Behavior and Water Status of Maize, Sorghum, and Tobacco under Field Conditions: I. At High Soil Water Potential. *Plant Physiology*, 51:31–36.

Turner, N. C. (1974). Stomatal Behavior and Water Status of Maize, Sorghum, and Tobacco under Field Conditions: II. At Low Soil Water Potential. *Plant Physiology*, 53(3):360–365.

Ulaby, F., Moore, R., and Fung, A. (1986). *Microwave remote sensing: active and passive. Volume I: microwave remote sensing fundamentals and radiometry.* Artech House, Boston, MA, USA.

Van Emmerik, T., Steele-Dunne, S. C., Judge, J., and Van De Giesen, N. (2015). Impact of Diurnal Variation in Vegetation Water Content on Radar Backscatter from Maize During Water Stress. *IEEE Transactions on Geoscience and Remote Sensing*, 53(7):3855–3869.

Van Emmerik, T., Steele-Dunne, S. C., Judge, J., and Van De Giesen, N. (2017). Dielectric Response of Corn Leaves to Water Stress. *IEEE Geoscience and Remote Sensing Letters*, 14(1):8–12.

Vanaja, M., Yadav, S. K., Archana, G., Jyothi Lakshmi, N. J., Ram Reddy, P. R., Vagheera, P., Abdul Razak, S. A., Maheswari, M., and Venkateswarlu, B. (2011). Response of C4 (maize) and C3 (sunflower) crop plants to drought stress and enhanced carbon dioxide concentration. *Plant, Soil and Environment*, 57(5):207–215.

Vermunt, P. C., Khabbazan, S., Bremer, K. S., Steele-Dunne, S. C., and Judge, J. (2019). Vegetation Water Content Dynamics in a Well-Watered Corn Canopy. *In preperation.*

Wiebe, H. and Prosser, R. (1977). Influence of Temperature Gradients on Leaf Water Potential. *Plant Physiology*, 59:256–258.

Yan, H., Wu, L., Filardo, F., Yang, X., Zhao, X., and Fu, D. (2017). Chemical and hydraulic signals regulate stomatal behavior and photosynthetic activity in maize during progressive drought. *Acta Physiologiae Plantarum*, 39(6).

Zarco-tejada, P. J., Hornero, A., Beck, P. S. A., Kattenborn, T., Kempeneers, P., and Hernández-clemente, R. (2019). Remote Sensing of Environment Chlorophyll content estimation in an open-canopy conifer forest with Sentinel-2A and hyperspectral imagery in the context of forest decline. *Remote Sensing of Environment*, 223(January):320–335.

Zhao, W., Sun, Y., Kjelgren, R., and Liu, X. (2015). Response of stomatal density and bound gas exchange in leaves of maize to soil water deficit. *Acta Physiologiae Plantarum*, 37(1).

A

Growth stages for corn

This table describes the BBCH scale that is used for the description of the development stages of corn, as described in the Crop Identification and BBCH Staging Manual: SMAP-12 Field Campaign (Earth Observation and Research Branch Team, 2011).

Figure A.1: Table with the different BBCH stages for corn (Earth Observation and Research Branch Team, 2011)

	0. Germination
00	Dry seed (caryopsis)
01	Beginning of seed imbibition
03	Seed imbibition complete
05	Radicle emerged from caryopsis
06	Radicle elongated, root hairs/side roots visible
07	Coleoptile emerged from caryopsis
09	Coleoptile penetrates soil
	1. Leaf Development[1]
10	First leaf through coleoptile
11	First leaf unfolded
12	2 leaves unfolded
13	3 leaves unfolded
1…	Stages continuous till …
19	9 or more leaves unfolded
	3. Stem Elongation[2]
30	Beginning of stem elongation
31	First node detectable
32	2 nodes detectable
33	3 nodes detectable
3…	Stages continuous till…
39	9 or more nodes detectable[1]
	5. Inflorescence Emergence, Heading
51	Beginning of tassel emergence, tassel detectable at top of stem
53	Tip of tassel visible
55	Middle of tassel emergence: middle of tassel begins to separate
59	End of tassel emergence: tassel fully emerged & separated
	6. Flowering, Anthesis
61	Male: stamens in middle of tassel visible Female: tip of ear emerging from leaf sheath
63	Male: beginning of pollen shedding Female: tips of stigmata visible
65	Male: upper & lower parts of tassel in flower Female: stigmata fully emerged
67	Male: flowering completed Female: stigmata drying
69	End of flowering: stigmata completely dry
	7. Development of Fruit
71	Beginning of grain development: kernels at blister stage, about 16% dry matter
73	Early Milk
75	Kernels in middle of cob yellowish-white (variety-dependent), content milky, about 40% dry matter
79	Nearly all kernels have reached final size
	8. Ripening
83	Early dough: kernel content soft, 45% dry matter.
85	Dough stage: kernels yellowish to yellow 55% dry matter
87	Physiological maturity: black dot/layer visible at base of kernels, 60% dry matter
89	Fully ripe: kernels hard & shiny, 65% dry matter
	9. Senescence
97	Plant dead & collapsing
99	Harvested product

[1] Tillering or stem elongation may occur earlier than stage 19; in this case continue with principal growth stage 3

[2] In maize, tassel emergence may occur earlier, in this case continue with principal growth stage 5.

B

Data forms fieldwork

Date:
Growth stage BBCH:
Stressed/Unstressed:
Weather conditions:

Comments:

Leaf Water Potential

Plant	Leaf[1]	Total leaves	Leaf height [cm]	collar height [cm]	Leaf direction[2]	S/NS[3]	Time: cutting	Time: LWP	Leaf water potential [PSI]	Leaf water potential [bar]	Comments

Figure B.1: The used field form for the leaf water potential measurements.

Date:
Growth stage BBCH:
Stressed/Unstressed:
Weather conditions:

Comments:

Stomatal conductance

Plant	Leaf[1]	Total leaves	Leaf height [cm]	Leaf direction[2]	S/NS[3]	Measured S/NS[3]	Cloud?	Time	Sample ID	T [°C]	Stomatal conductance [mmol/(m^2s)]	Comment

Figure B.2: The used field form for the stomatal conductance measurements.

C

Leaf shading

During the stomatal conductance measurements, notion was made of the shading of the measured leaves. The tables below show the percentage per leaf for different observed shading. The growing season was divided into the following groups:

- Beginning of the vegetative stage: 26 April - 18 May (BBCH 13 - 19)
- End of the vegetative stage: 19 May - 31 May (BBCH 30 - 59)
- Reproductive stage: 1 June - 11 June (BBCH 61 - 71)

Table C.1: Shading of the measured leaves in the beginning of the vegetative stage: 26 April - 18 May (BBCH 13 - 19)

Leaf	Total plants	Shaded	Sunny	Partially shaded				Clouded	After sunset	Not noted
				Total	*Shaded*	*Sunny*	*Unknown*			
13	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-
9	7	-	42.86%	-	-	-	-	57.14%	-	-
7	50	2.00%	76.00%	6.00%	*2.00%*	*4.00%*	-	8.00%	14.00%	-
6	13	-	92.31%	7.69%	-	-	*7.69%*	-	-	7.69%
5	74	8.11%	68.92%	35.14%	*6.76%*	*20.27%*	*8.11%*	5.41%	9.46%	8.11%
4	33	-	72.73%	36.36%	-	*9.09%*	*27.27%*	-	-	27.27%
3	102	17.65%	64.71%	20.59%	*7.84%*	*9.80%*	*2.94%*	-	6.86%	10.78%
2	52	11.54%	63.46%	9.62%	-	-	*9.62%*	-	-	25.00%
1	36	16.67%	58.33%	2.78%	-	-	*2.78%*	-	-	25.00%

Table C.2: Shading of the measured leaves in the end of the vegetative stage: 19 May - 31 May (BBCH 30 - 59)

Leaf	Total plants	Shaded	Sunny	Partially shaded				Clouded	After sunset	Not noted
				Total	*Shaded*	*Sunny*	*Unknown*			
13	7	14.29%	85.71%	42.86%	*14.29%*	*28.57%*	-	-	-	-
11	76	7.89%	71.05%	27.63%	*6.58%*	*21.05%*	-	21.05%	-	-
9	76	23.68%	55.26%	47.37%	*22.37%*	*25.00%*	-	21.05%	-	-
7	71	25.35%	52.11%	49.30%	*16.90%*	*32.39%*	-	22.54%	-	-
6	-	-	-	-	-	-	-	-	-	-
5	80	65.00%	12.50%	35.00%	*25.00%*	*10.00%*	-	22.50%	-	-
4	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-	-	-

Table C.3: Shading of the measured leaves in the end of the reproductive stage: 1 June - 11 June (BBCH 61 - 71)

Leaf	Total plants	Shaded	Sunny	Partially shaded				Clouded	After sunset	Not noted
				Total	*Shaded*	*Sunny*	*Unknown*			
13	67	8.96%	79.10%	26.87%	*4.48%*	*22.39%*	-	11.94%	-	-
11	67	26.87%	59.70%	56.72%	*17.91%*	*38.81%*	-	13.43%	-	-
9	67	52.24%	34.33%	65.67%	*31.34%*	*34.33%*	-	13.43%	-	-
7	44	65.91%	13.64%	38.64%	*25.00%*	*13.64%*	-	20.45%	-	-

D

Diurnal stomatal conductance measurements

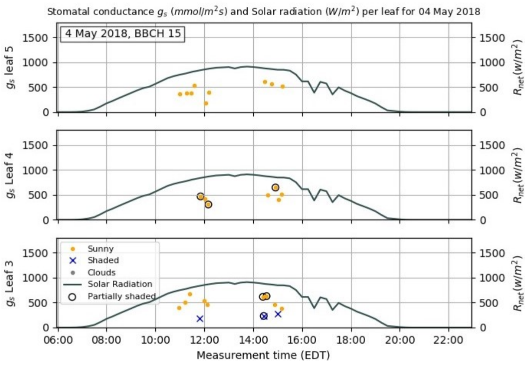


Figure D.1: Stomatal conductance observations for 4 May 2018.

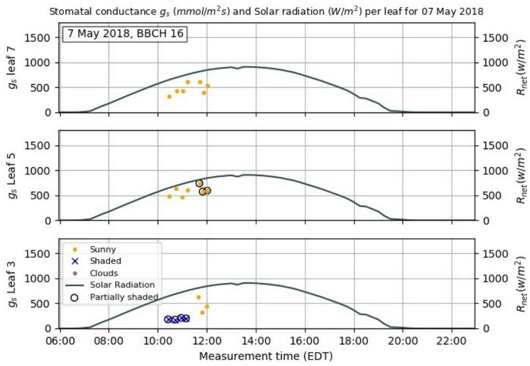


Figure D.2: Stomatal conductance observations for 7 May 2018.

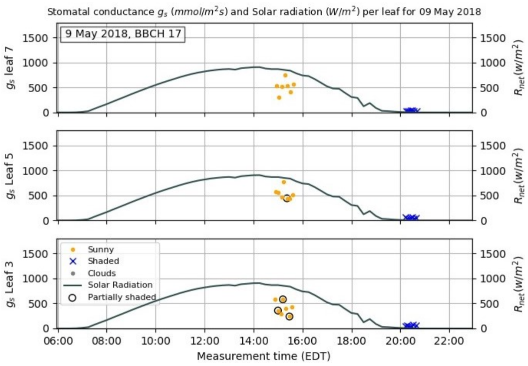


Figure D.3: Stomatal conductance observations for 9 May 2018.

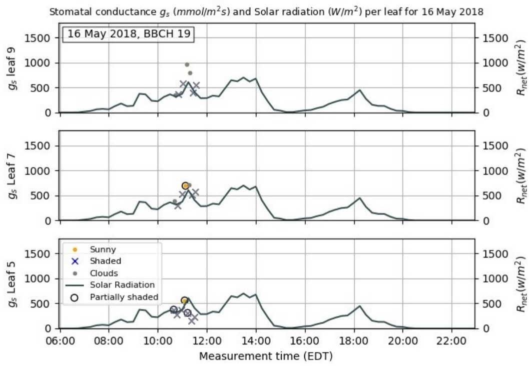


Figure D.4: Stomatal conductance observations for 16 May 2018.

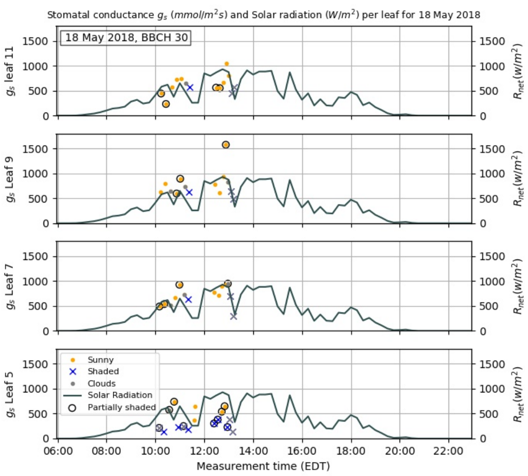


Figure D.5: Stomatal conductance observations for 18 May 2018.

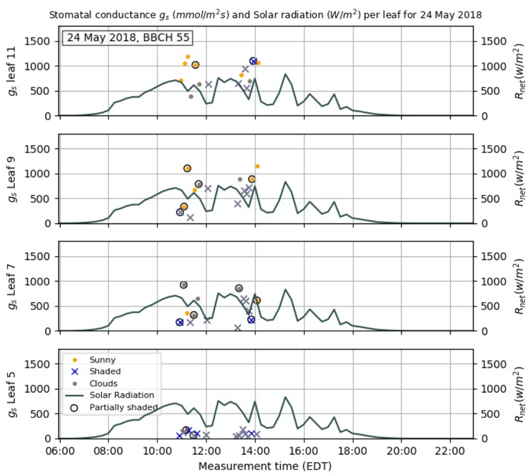


Figure D.6: Stomatal conductance observations for 24 May 2018.

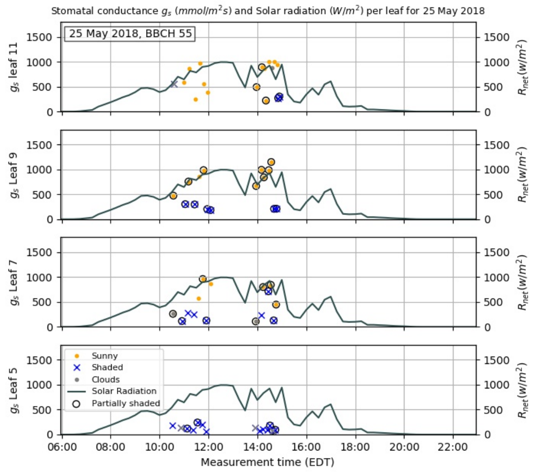


Figure D.7: Stomatal conductance observations for 25 May 2018.

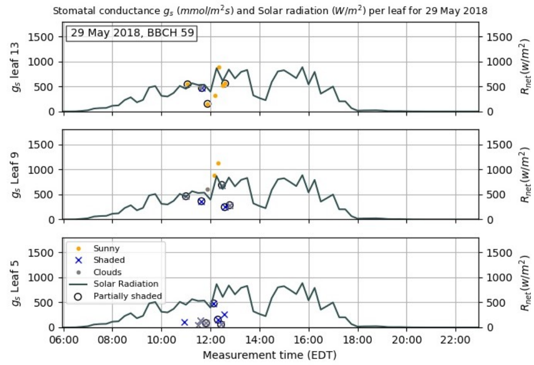


Figure D.8: Stomatal conductance observations for 29 May 2018.

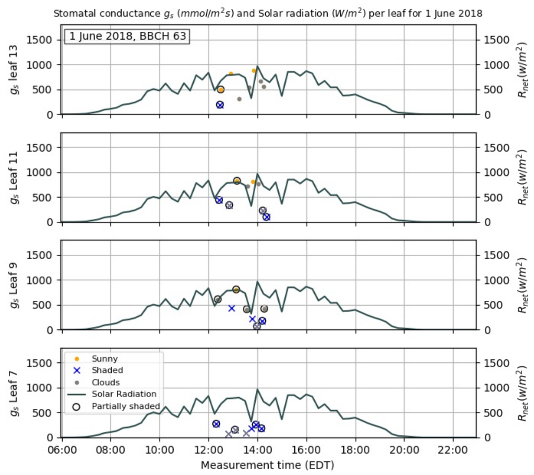


Figure D.9: Stomatal conductance observations for 1 June 2018.

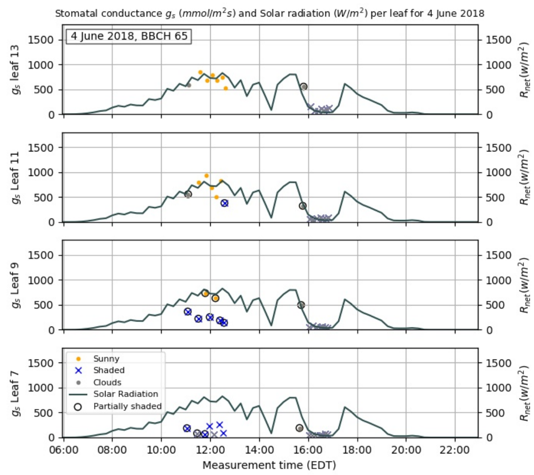


Figure D.10: Stomatal conductance observations for 4 June 2018.

E

Leaf orientation

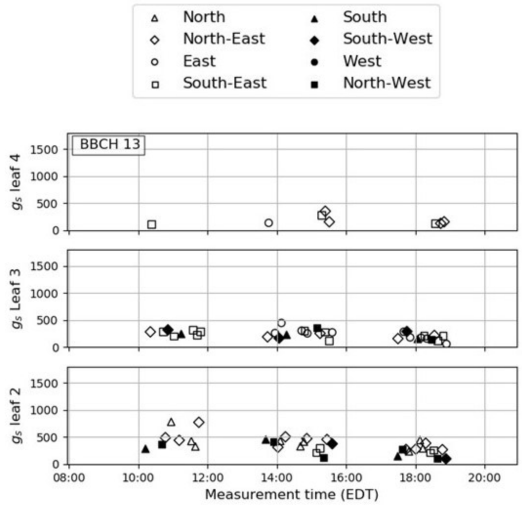


Figure E.1: Stomatal conductance ($mmol/m^2s$) observations per leaf orientation for 26 April 2018.

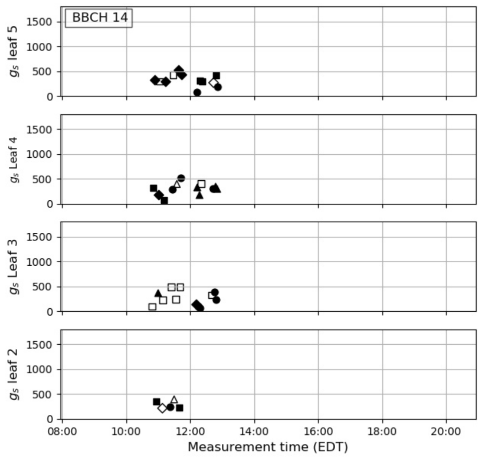


Figure E.2: Stomatal conductance ($mmol/m^2s$) observations per leaf orientation for 2 May 2018.

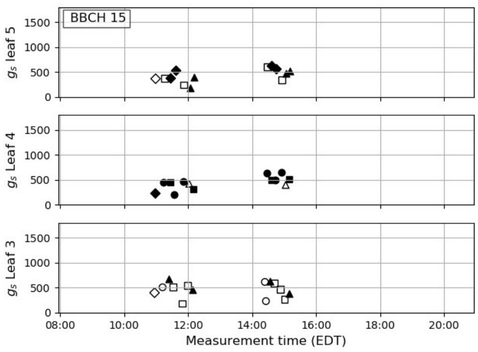


Figure E.3: Stomatal conductance ($mmol/m^2s$) observations per leaf orientation for 4 May 2018.

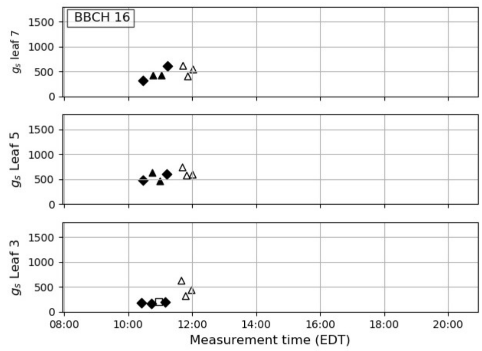


Figure E.4: Stomatal conductance ($mmol/m^2s$) observations per leaf orientation for 7 May 2018.

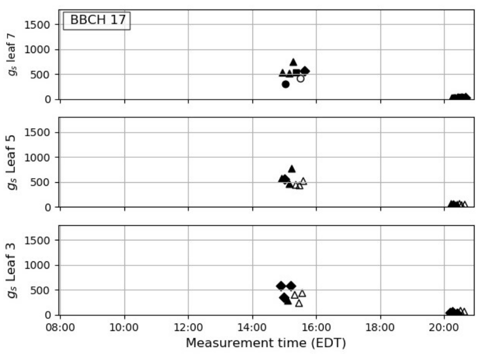


Figure E.5: Stomatal conductance ($mmol/m^2s$) observations per leaf orientation for 9 May 2018.

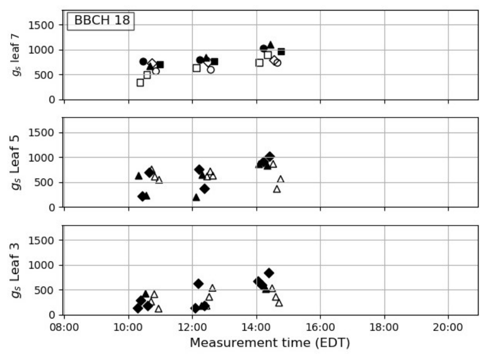


Figure E.6: Stomatal conductance ($mmol/m^2s$) observations per leaf orientation for 11 May 2018.

F

Leaf temperature

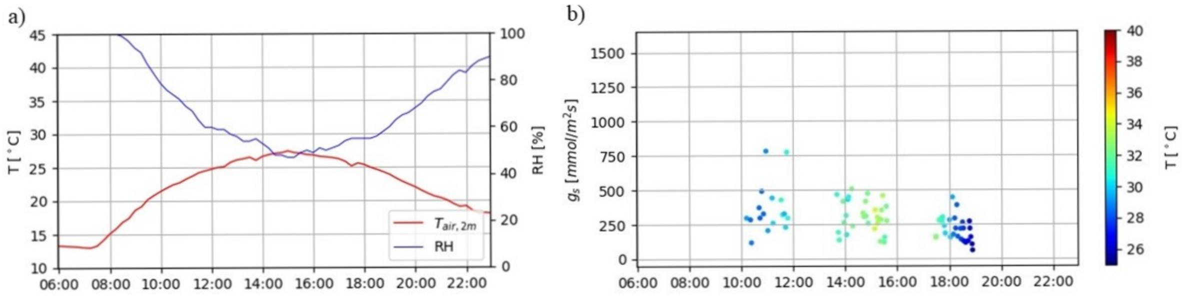


Figure F.1: 26 April 2018 a) Relative humidity (%) and air temperature ($^\circ C$) at 2m, b)Stomatal conductance ($mmol/m^2s$) colored by leaf temperature ($^\circ C$)

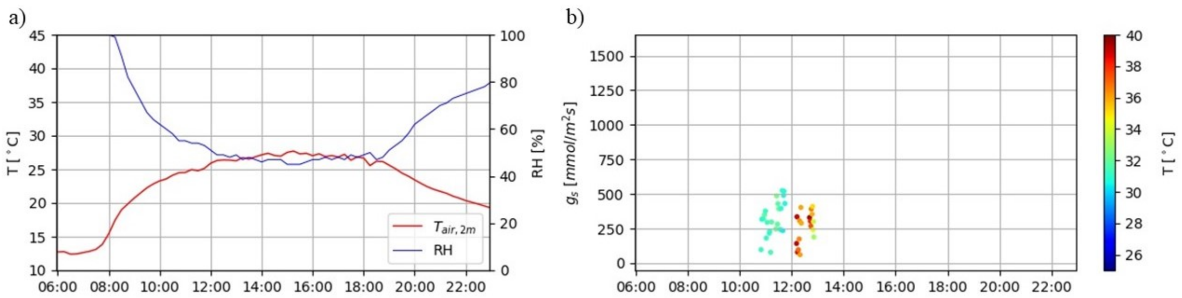


Figure F.2: 2 May 2018 a) Relative humidity (%) and air temperature ($^\circ C$) at 2m, b)Stomatal conductance ($mmol/m^2s$) colored by leaf temperature ($^\circ C$)

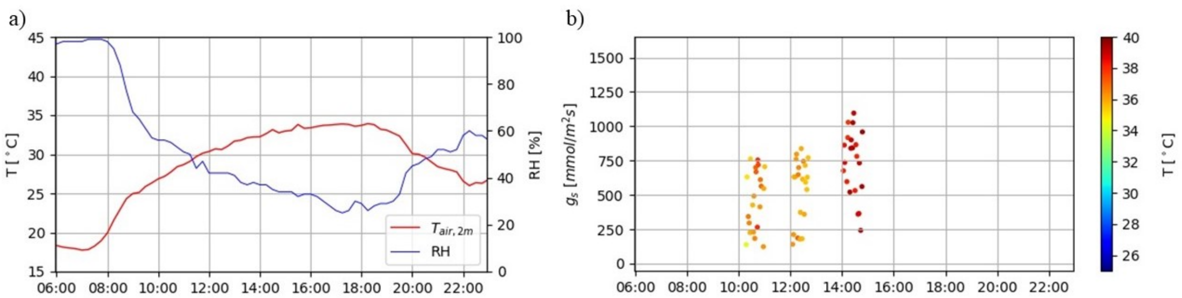


Figure F.3: 11 May 2018 a) Relative humidity (%) and air temperature ($^\circ C$) at 2m, b)Stomatal conductance ($mmol/m^2s$) colored by leaf temperature ($^\circ C$)

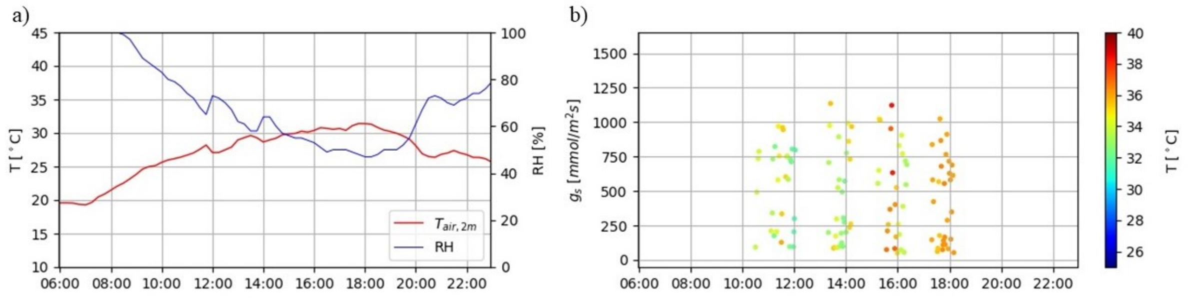


Figure F.4: 23 May 2018 a) Relative humidity (%) and air temperature ($°C$) at 2m, b)Stomatal conductance ($mmol/m^2s$) colored by leaf temperature ($°C$)

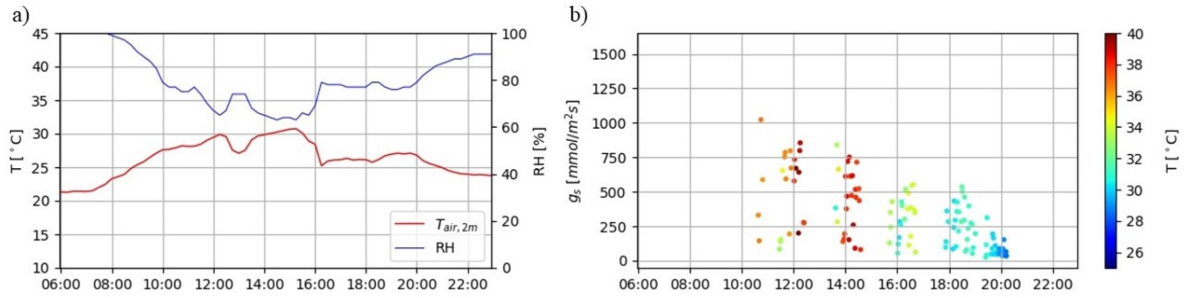


Figure F.5: 11 June 2018 a) Relative humidity (%) and air temperature ($°C$) at 2m, b)Stomatal conductance ($mmol/m^2s$) colored by leaf temperature ($°C$)

G

Leaf water potential differences over height

Table G.1 shows the average difference in measured leaf water potential ψ_l between the measured leaves, and therefore over height. The bold numbers indicate a decrease that is larger than two times the measurement accuracy. The red numbers indicate the cases where for each individual plant the difference between the leaves was larger than two times the measurements accuracy.

Table G.1: The average difference in leaf water potential over the different measured leaves.

Measurement time	ψ_{l5}-ψ_{l3}	ψ_{l7}-ψ_{l5}	ψ_{l9}-ψ_{l5}	ψ_{l9}-ψ_{l7}	ψ_{l11}-ψ_{l9}	ψ_{l13}-ψ_{l9}	ψ_{l13}-ψ_{l11}
09-05-2018 06:00	0.080	**-0.027**					
09-05-2018 07:40	**-0.013**	0.027					
09-05-2018 12:34	**-0.013**	**-0.010**					
09-05-2018 13:45	0.107	**-0.047**					
09-05-2018 18:02	0.103	**-0.150**					
09-05-2018 19:30	-0.020	**-0.093**					
11-05-2018 06:08	**-0.023**	**-0.057**					
11-05-2018 07:42	**-0.023**	**-0.010**					
14-05-2018 06:10		0.050		**-0.053**			
14-05-2018 08:05		**-0.010**		**-0.013**			
16-05-2018 06:28		**-0.047**		**-0.023**			
16-05-2018 08:53		0.103		**-0.023**			
16-05-2018 18:53		**-0.010**		0.023			
16-05-2018 20:30		**-0.047**		0.000			
18-05-2018 06:17		**-0.010**		**-0.023**			
18-05-2018 07:53		**-0.027**		0.000			
21-05-2018 06:11		**-0.037**		**-0.037**	0.013		
21-05-2018 08:16		**-0.013**		0.000	0.000		
23-05-2018 06:18		**-0.027**		**-0.070**	**-0.033**		
23-05-2018 08:30		0.010		**-0.033**	**-0.033**		
23-05-2018 18:38		**-0.010**		**-0.067**	**-0.013**		
23-05-2018 20:20		**-0.060**		0.000	**-0.080**		
25-05-2018 06:18		0.010		0.010	**-0.010**		
25-05-2018 08:12		**-0.043**		0.020	**-0.033**		
29-05-2018 06:18			**-0.023**			0.010	
29-05-2018 08:22			**-0.107**			**-0.043**	
30-05-2018 06:21			**-0.037**			0.000	
30-05-2018 08:10			**-0.037**			-0.023	
01-06-2018 06:24				0.037	**-0.027**		**-0.010**
01-06-2018 09:16				**-0.050**	0.013		**-0.070**
04-06-2018 06:32				0.023	0.000		0.013
04-06-2018 09:22				0.000	**-0.023**		**-0.033**
04-06-2018 18:24				0.043	**-0.023**		**-0.020**
04-06-2018 20:26				0.057	**-0.010**		**-0.073**
06-06-2018 06:18				0.990	-0.006		**-0.050**
08-06-2018 06:10				**-0.210**	0.095		**-0.035**
08-06-2018 13:10				**-0.033**	0.088		**-0.070**
08-06-2018 18:30				**-0.047**	-0.078		0.000
11-06-2018 06:14				0.070	**-0.010**		**-0.013**

H

Leaf water potential sample leaf length

Table H.1: Leaf length measurements on 11 May 2018 (BBCH 18)

Plant	Leaf	Sample length (cm)	$t_{fieldremoval}$	t_{cut}	$t_{measurement}$	ψ_l (MPa)
3	7	24	07:42	08:36	08:49	-0.31
3	7	15	07:42	08:36	08:43	-0.38
3	7	12	07:42	08:36	08:57	-0.52

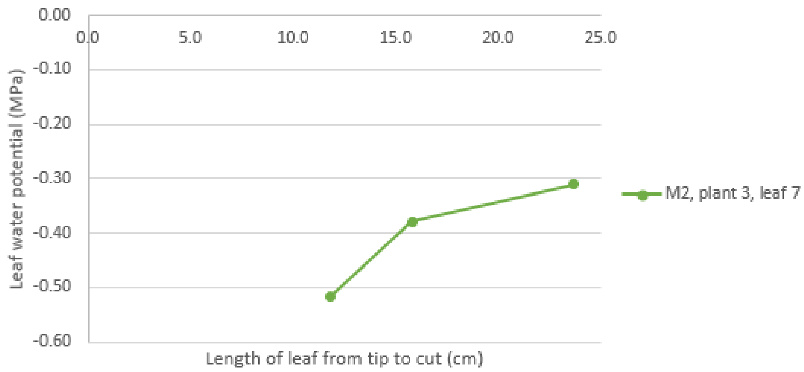


Figure H.1: Results leaf length measurement on 11 May 2018 (BBCH 18)

Table H.2: Results of the first leaf length measurement on 14 May 2018 (BBCH 19)

Plant	Leaf	Sample length (cm)	$t_{fieldremoval}$	t_{cut}	$t_{measurement}$	ψ_l (MPa)
1	7	45	06:10	06:25	06:45	-0.12
1	7	30	06:10	06:45	06:50	-0.17
3	9	42	06:10	07:18	07:34	-0.34
3	9	30	06:10	07:35	07:38	-0.38
1	7	59	08:05	08:20	08:26	-0.14
1	7	25	08:05	08:31	08:32	-0.21
2	7	59	08:05	08:36	08:47	-0.21
2	7	30	08:05	08:50	08:54	-0.34

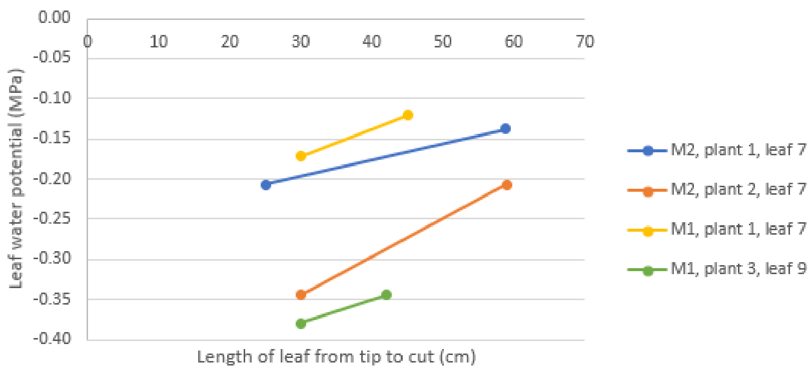


Figure H.2: Results leaf length measurement during measurements at 6:00 (M1) and 8:00 (M2) on 14 May 2018 (BBCH 19)

Table H.3: Results of the second leaf length measurement on 14 May 2018 (BBCH 19), on leaf 8 of three different plants

Plant	Leaf	Sample length (cm)	$t_{fieldremoval}$	t_{cut}	$t_{measurement}$	ψ_l (MPa)
A	8	50	09:55	10:06	10:12	-0.55
A	8	40	09:55	10:14	10:19	-0.62
A	8	35	09:55	10:20	10:24	-0.69
A	8	30	09:55	10:25	10:28	-0.72
A	8	25	09:55	10:30	10:34	-0.79
B	8	50	09:55	10:35	10:38	-0.69
B	8	40	09:55	10:40	10:44	-0.76
B	8	35	09:55	10:45	10:52	-0.79
B	8	30	09:55	10:54	10:58	-0.76
B	8	22	09:55	11:01	11:04	-0.76
C	8	45	09:26	09:26	09:28	-0.21
C	8	35	09:26	09:29	09:32	-0.24
C	8	25	09:26	09:33	09:37	-0.34
C	8	15	09:26	09:39	09:44	-0.55

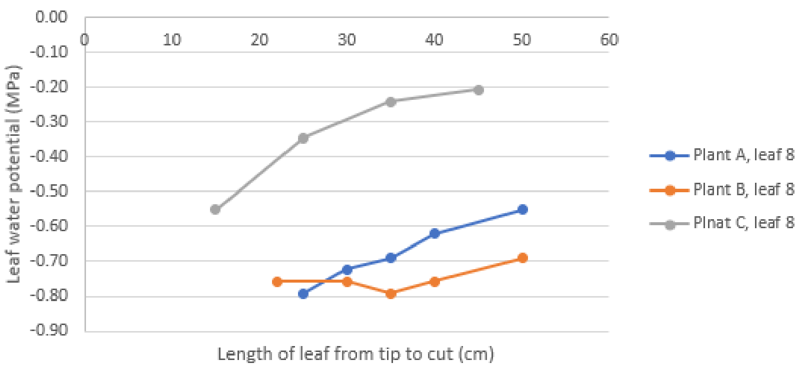


Figure H.3: Results of the second leaf length measurement on 14 May 2018 (BBCH 19), on leaf 8 of three different plants

Table H.4: Leaf length measurements on 18 May 2018 (BBCH 30)

Plant	Leaf	Sample length (cm)	$t_{fieldremoval}$	t_{cut}	$t_{measurement}$	ψ_l (MPa)
1	7	45	06:17	06:41	06:48	-0.17
1	7	20	06:17	06:59	07:01	-0.28

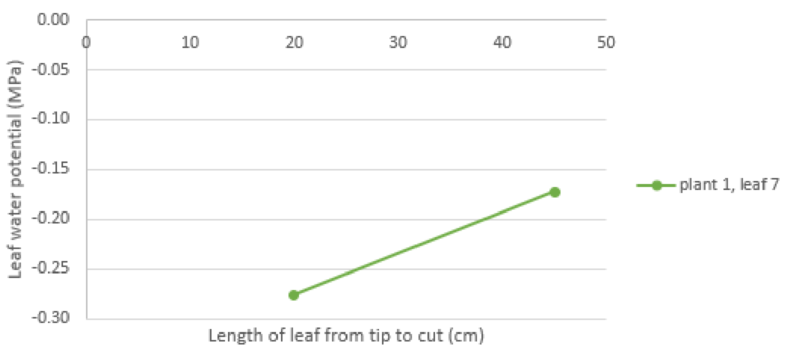


Figure H.4: Results leaf length measurement on 18 May 2018 (BBCH 30)

Additional Sap Flow results

Below, several additional sap flow results are shown for days with various solar radiation and soil water availability. On 5 June, hardly any cloud cover was observed. The sap flow shows the same shape as is expected from the stomatal conductance on a day with no limitation in solar radiation. On 6 June, there was heavy cloud cover, therefore hardly any sap flow was observed. On the 8th of June, the sap flow decreases as a heavy cloud blocks the solar radiation at 13:00. After this, the sap flow stays low. This is likely caused by the limited solar radiation, as another cloud passes over before the sap flow had fully recovered. However, this day was one of the days with a low soil water potential, this could also have limited the stomatal conductance in the afternoon. On 10 June, the soil water potential had been low for four days. Of all the days in the growing season, it was most likely that the plants experienced water stress during this day. At 12:00 in the afternoon, a heavy front came over the field, causing a stop in the sap flow. During most moments with low solar radiation, the sap flow had low values, however, here the sap flow was not detected at all. During this time of limited solar radiation, a heavy rainfall event took place, increasing the soil water potential.