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Second Cycle Degree (MSc) In Sustainable Agriculture

Hailstorm effects on leaf area index (LAI) and chlorophyll, anthocyanin, and flavonoid pigments in winter wheat (*Triticum aestivum* L.)

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Summary

Abstract

Hailstorms may seriously harm crops, resulting in yield losses and eventually worsening the financial situation of farmers. The crop's growth stage at which damage occurs may have an influence on the yield damage degree as well. Hail damages can result in stem breakings that prevent nutrients from moving towards the spikes in winter wheat (*Triticum aestivum* L.), as well as defoliation and direct grain loss, thus reducing the final yield output. Defoliation, may results in detectable leaf area index decreases, as well as plant pigment changes caused by the injuries. Considering the spatial and intensity extent that these weather events can reach, and the difficulties of precise on-site damage assessment by insurance field inspectors, there is a need to find suitable vegetation traits that can help to better define damage for remote sensing upscaling. During the 2022 cropping season, this study was conducted at the University of Padova's experimental farm "L. Toniolo" in Legnaro (Northern Italy), to explore the effects of hailstorms on the leaf area index, chlorophyll content, anthocyanins, and flavonoids on winter wheat at different developmental stages (flowering, milky and over-ripe). Four damage intensities (0%, 20%, 50%, and 80%) were simulated in three replicates during different plant developmental stages using specifically built prototypes. The study compared two techniques for calculating LAI: destructive and non-destructive, considering the crucial significance of LAI as a proxy measure for vegetation state and modeling. The utility and dependability of the indirect measurements made with a ceptometer (non-destructive) were confirmed by the generally good agreement with the direct (destructive) LAI measurements. The crop was then monitored across the May-July span of the cropping season. When wheat was severely damaged by hail, there was a general decrease in LAI and chlorophyll content following damage. The latter, though, showed a distinct behavior at the end of the season, with increase chlorophyll concentration in the most damaged plants. Anthocyanins and flavonoids generally increased following damage across all plant stages. The yield damage degree was mostly influenced by the damage intensity, with the 80% treatment resulting in an average yield drop of 55% in comparison to the control across all the considered stages.

1 Introduction

1.1 Hailstorms and climate change

Extreme weather phenomena such as storms, heat waves, and droughts have become more common in recent decades. During the summers of 2003 and 2010, Europe and several portions of Russia saw significant heat waves. During the summer of 2007, the United Kingdom endured a series of severe floods of unprecedented proportions that struck Germany, Hungary, and other countries in 2013 (Vescovo et al., 2016). Climate change may increase the frequency and intensity of these weather extremes, thus harming the economy as a whole. According to the IPCC special report, global average temperatures have risen by around 1 °C since the pre-industrial period, and human warming adds about 0.2 °C to world average temperatures every decade (IPCC 2018). At the present rate of anthropogenic greenhouse gas (GHG) emissions, the research predicts that global average warming will exceed 1.5 °C between 2030 and 2052 (Ogunbode et al., 2020). In the absence of severe climate policy agreements, emissions are likely to grow significantly, particularly as a result of fast economic expansion in emerging nations (Botzen et al., 2010). Few studies have been conducted to investigate the relationships between climatic indicators and insured hailstorm damage. For example, in France and Switzerland, Dessens (1995) and Willemse (1995) find direct relationships between temperature and hailstorm damage, suggesting that global warming will increase hailstorm losses in the future.

One of the most pressing concerns in the twenty-first century is providing adequate food for the rising population while preserving the already stressed environment. The challenge that climate change brings to global food security makes this effort much more critical (Kang et al., 2009). To satisfy these demands, there is an urgent need to address the core reason for the decline in food production, so that agriculture's full potential may be realized. Hailstorm damage to crops is one of these root cause concerns (Bal et al., 2014). Hail represents a serious threat to crops worldwide, harming food security as well as the economy of the agriculture sectors. Hail is caused by deep convective storms with powerful updrafts, high supercooled liquid water content, high cloud tops, and sufficient longevity of the phenomena. Hailstorms are often highly structured convective systems, such as multicell, mesoscale convective systems, or supercells. Hail is generally defined as ice stones having a diameter of at least 5 mm (Punge & Kunz, 2016). To properly analyze the hail phenomenon, it is necessary to acquire a large amount of information due to the significant variations in hail conditions that result in agricultural damages (Changon et al., 2009). There are several distinct climatic conditions in which hailstorms might occur. The atmospheric circumstances are unfavorable in too-cold or too-warm regions. In cold climates, the shallowness of cumulus clouds prevents hailstorm formation, whereas, in warm climates, the presence of warm air aloft reduces the likelihood of hailstorm development (Baldi et al., 2014). Hailstorms do happen in the majority of European countries, in Italy they happen more frequently in the northern portion of the country, nonetheless, severe incidents have also been recorded in Lazio and Campania in the Central-South region (Baldi et al., 2014). These occurrences frequently result in injuries and/or major damage to agriculture, automobiles, and structures.

1.2 General agriculture harm

Extreme events can take many distinct forms, which causes their influence on many components of the agricultural growth cycle and related field management to vary. The timing and circumstances of field activities can also be impacted by extreme weather events, which can also have an adverse effect on the physiological processes of plants and cause direct physical harm to crops. For instance, high temperatures and low precipitation levels that cause heat and drought stress can have a negative impact on crop photosynthesis and transpiration and raise the risk of pest and disease outbreaks (van der Velde et al., 2012).

1.3 From broad to narrow. Hailstorm impacts on winter wheat

Windblown hailstones with a diameter of 1 cm or greater can inflict significant damage to wheat stems. Hail damage is a significant concern for agriculture (Dávila et al. 1985), causing economic and social challenges (Sánchez et al., 1996). Climate change is the principal source of altering weather event frequency, which leads to a drop in agricultural yields across a number of crops, including essential cereal crops like wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), and rice (*Oryza sativa* L.) (Botzen et al., 2010). In the case of maize, the severity of damages resulting from hailstorms appear to be connected to the plant stage at which they occur, although information concerning winter wheat is limited (Shekoofa et al., 2012).

The yield damage has been connected to hailstorm indicators such as the quantity of hailstones, maximum diameter, mass, velocity, and kinetic energy of hailstones determined from hailpads. Crop damage was directly connected to the frequency of hailstorms with larger diameters and the energy delivered by complete hailfall (Changnon, 1971). The severity of crop damage was shown to be rather well connected with impact energy (kinetic energy), i.e., the mass and quantity of hailstones with diameters more than 0.64 cm (Omoto & Seino, 1978). As a result, heavy gusts accompanying hail easily force the thick wheat plants over, and in this configuration, they tend to partially screen each other from wind-blown hail due to their stem density (Changnon, 1971).

On wheat, the largest decrease normally happens when the spike is damaged during the milk stage, therefore causing direct grain loss (Ferguson et al., 1987). The hail damage to barley, a crop comparable to wheat, is said to be highest when it happens during the heading or soft dough phases (Sánchez et al., 1996). Moreover, injuries caused by hail damage may also make it easier for some plant to develop infections, reducing production and quality even more (Robertson et al., 2011).

Commonly, insurance firms rely on inspectors for damage assessment. The damage assessment process can be labor- and time-intensive, but it can also be spatially constrained in the case of large fields (de Leeuw et al., 2014). Knowing how hailstorm damage affects crops and how their vegetational traits change during the cropping season following the damage might be critical for more accurate damage estimations and remote sensing applications (Jelić et al., 2020). The traditional damage assessment process applied by an insurance field inspector works with the selection of representative plants in various spots across a field and standardized chart to estimate real plant damage and crop loss based on defoliation, direct grain loss, and stem breakings. However, precisely evaluating damage and its geographic variations within a field might take time. Due to geography, wind patterns, and the unpredictability of damage intensity in a storm, damage frequently occurs in uneven patterns and varies across a field (Erickson et al., 2004).

1.4 Leaf Area Index (LAI)

According to Watson (1947), the Leaf area index (LAI) represents the entire one-sided area of leaf tissue per unit of ground flat surface area. This definition implies that LAI is a dimensionless integer describing the canopy of an ecosystem. Because it affects the microclimate both inside and outside the canopy, as well as canopy water interception, radiation extinction, and the exchange of water and carbon dioxide, leaf area is crucial for biogeochemical cycles in ecosystems. Stand production changes in response to changes in the canopy leaf area (caused by frost, storm, defoliation, drought, or management practices) (Bréda, 2003). LAI has been identified as a key climatic variable by the global climate change research community as a basic feature of global vegetation (Fang et al., 2019). There is a greater capability for a canopy to absorb photosynthetically active radiation (PAR) from the sun the more leaf material it has. Since LAI functions as a bridge to accelerate the rate of leaf biophysical and biogeochemical processes, such as leaf photosynthesis and stomatal conductance at the canopy level, it is also utilized as a metric of the lifecycle events of plants (Niu et al., 2011). This considered, the canopy structure of crops is significantly impacted by hailstorms, which can have a direct impact on the leaf area. Due to defoliation and stem injury, LAI decreases as hail damage rises. For instance, hail damage alters the total leaf area by resulting in bent and broken plant stems, crushed canopies, increased lodging, and defoliation, which eventually results in a decrease in crop output and/or quality (Counce et al., 1994).

LAI can be measured by direct and indirect methods. Destructive strategies, litter catchers, and allometric techniques are some of the direct methods. The destructive sample and measure of the leaf area is referred to as the destructive technique. "True LAI" is the common name for it. Direct analysis is done by harvesting and measuring of each leaf area to obtain a direct measurement of LAI. Although flatbed scanners have increased the efficiency of this procedure, it is still labor-intensive, time-consuming, and implies the removal of plant material. As a result of the actual measurement of each leaf, it does, however, continue to be the approach with the highest level of accuracy for computing the leaf area index. Another method of measuring LAI directly is using litter traps, although they are ineffective in evergreen canopies and can only collect data from leaves that have died and been removed from the plant (Campbell, 2020). This being considered, indirect measurements have the advantage of reducing measurement time and avoid destructive sampling. Measuring related variables, such as the quantity of light that passes through or is reflected by a canopy, can be used to estimate LAI. Several instruments have been developed for this purpose, e.g., the ceptometer is a device that monitors photosynthetically active radiation (PAR) above and below the canopy and calculates LAI based on the ratio of the two. Aside from theoretical simplifications, the main factors influencing the accuracy of ceptometer measurements are the plant species-specific leaf angle distribution, and foliage clumping (Pokovai & Fodor, 2019).

1.5 Influence of hail on chlorophyll

Chlorophyll is engaged in all aspects of photosynthesis's key events (Katz et al., 1978). According to Macedo et al. (2007), the lack of substantial impacts of any defoliation treatment on any of the chlorophyll fluorescence indicators tested shows that differences in photosynthesis of wounded leaves are most likely due to the varied spatial defoliation patterns. The geographical pattern of wheat defoliation influences photosynthetic and other gas exchange reactions.

The study discovered considerably higher stomatal conductance produced by defoliation and its relationship with time following defoliation on the wounded leaf's surviving tissues. The stomatal conductance was higher after 24 hours from defoliation compared to the results after one hour. Defoliation, on the other hand, had no effect on photosynthesis in non-damaged leaves, while stomatal conductance values were greater in wounded leaves, suggesting that defoliation affected photosynthesis (Macedo et al., 2007). Hailstorms can also bruise stems following the impact, which frequently crushes vascular tissues and obstructs water and solute transport through the plant. When water is blocked, it causes drought stress, which can lower the chlorophyll content of leaves and decrease photosynthetic activity, e.g. in winter wheat (Bijanzadeh & Emam, 2010).

1.6 Influence of hail on other plant pigments

Plant pigments are categorized into numerous groups: chlorophylls, carotenoids (carotenes, xanthophylls), flavonoids (chalcones, anthocyanins, flavones, flavonols), and betalains (betaxanthin, betacyanin) (Ewa, 2009). The primary pigment classes are found in many plant organs. Flavonoids may be found in practically every tissue, including leaves, roots, seeds, fruits, and flowers.

Flavonoids are a class of plant polyphenolic secondary metabolites with a three-ring chemical structure (C6–C3–C6). Flavonoids are classified into four types: anthocyanins (red to purple pigments), flavonols (colorless to pale yellow pigments), flavanols (colorless pigments that become brown during oxidation), and proanthocyanidins (PAs) or condensed tannins. These chemicals are broadly disseminated in varying levels depending on the plant species, organ, developmental stage, and growth circumstances (Petrussa et al., 2013). In plants, flavonoids have important roles in pigmentation and auxin transport control, UV protection, defense against pathogens and pests, pollen fertility, and defense against diseases and pests. More than ten thousand different types of flavonoids have now been discovered (Guo et al., 2008).

Flavonoids protect plants against biotic and abiotic stresses, have a wide range of biological activities, and play a significant role in the plant-environment interaction.
Anthocyanins are synthesized from a branch of the flavonoid system, and their production is the most researched secondary metabolic process in plants, being one of the most prevalent groups of natural pigments in the plant kingdom. They are present in all plant tissues and exhibit a wide range of colors, spanning almost the whole visible spectrum, from orange and red to purple and blue tones (Winkel-Shirley, 2002). Anthocyanins, which have strong antioxidant characteristics, protect plants against a variety of biotic and abiotic stressors.

1.7 Aims of the thesis project

The goals of this research are two-folded:

1) To characterize winter wheat response to different degrees of hailstorm damage by analyzing leaf area and pigments content.
2) To compare different LAI estimation techniques, namely destructive and non-destructive methods, and their applicability in damaged canopies.

2 Methods and materials

2.1 Experimental site and experimental design

The experiment was conducted at the "L. Toniolo" University of Padova's experimental farm, sited in Legnaro, Agripolis campus (Northern Italy). The data was collected on winter wheat field from the end of April 2022 to the end of August 2022. During the 2022 farming season, the experiment was conducted on winter wheat (*Triticum aestivum* L.). Hail damages were simulated. Hail damage treatments were done at three different levels, i.e., 0% 20%, 50%, and 80% of leaf inefficiency in three replicates for each degree of damage. Leaf inefficiency is a parameter commonly used during the damage assessment by insurance field inspectors. It assesses the degree of removed and shredded leaves as well as broken leaf ribs. These damages are scored by field inspectors to determine the final level of damage, since damaged leaves are thought to contribute less to photosynthesis on a scale based on the severity of the damage. Another parameter considered is the complete removal or partial damage of the spike kernels. As a result, throughout the reproductive development phases following blooming, the rate of damage evaluation focuses also on the number of kernels lost as well as broken stems. Total, 36 plots were designed for wheat, in which hail damage was done in 27 plots at various phases of development, as described by the BBCH scale (Lorentz, D., 1994). In wheat, when the crop was fully blooming (61-69 BBCH scale) and 50% of the anthers were developed, the first damage was done. When the grains reached the milky stage and achieved their ultimate size but remained green, the second damage was applied (73-77 BBCH scale). The third damage was performed when the grain had become hard and could not be dented with a thumbnail (92 BBCH scale). The remaining three plots were designated as control plots and received no treatment. A sample point (A) was chosen inside each plot to monitor several characteristics such as LAI, crop chlorophyll content, anthocyanin, and flavonoid pigments. Additional sampling sites were also chosen, and their names were (B, C, and D) (fig.1).

Figure 1: Experimental site of wheat in Legnaro's Agripolis (Northern Italy). Plot letters designate the stage (Flowering stage (F), Milky stage (ML), Over-ripe stage (MP), and the type of harm (Hail Damage (G), Wind Damage (V)). Control Plots are designated as (X). The percentage of damage is shown by (20, 50, and 80).

2.2 Hail damage simulation

The hail damage was carried out using two prototypes (fig. 2) developed at the University of Padova experimental farm “L. Toniolo”. The prototype, which may be attached to a tractor, is made up of a 6 m wide steel framework that supports a hydraulic motor that permits the rotation of a metal pipe that holds a succession of nylon ropes that produce damage by striking the foliage at varying speeds. This system enables varied levels of damage to be created by varying the working height and rotation speed of the tube.

Figure 2: the first prototype while doing simulations of hail damage in winter wheat.

2.3 Field operations

The sequence of field activities carried out in wheat crop during the experiment is reported in fig. 3. Damages were performed on a specific date that corresponded to the wheat's desired development stages, while surveys were performed 7 to 10 days later (depending on weather conditions) leaving a time interval between the damage and the measurement to allow the plant to develop a response to the treatment over time, such as a pigmentation change or spike/leaf necrosis after the damage.

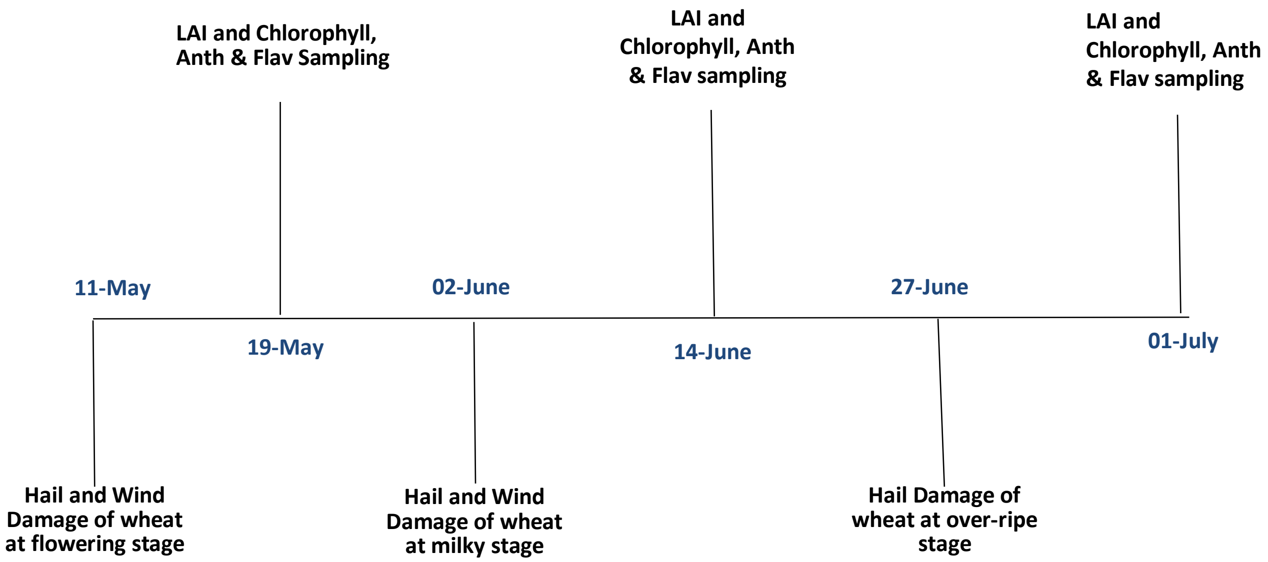

Figure 3: The field activities conducted during 2022 cropping season

2.4 Indirect Method for measuring LAI

The leaf area index was computed using an LP-80 ceptometer (Decagon Devices Inc., Pullman, WA, USA). The device is an indirect and passive sensor that detects light interception in plant canopies and calculates the leaf area index using an array of 80 sensors organized in a measurement rod. Equation 1 is employed by the instrument to calculate LAI when the equipment detects PAR (photosynthetically active radiation) above and below the canopy.

$$LAI = \frac{\left[\left(1-\frac{1}{2k}\right)fb-1\right]ln\tau}{A(1-0.47)fb} \quad (1)$$

Where k is the extinction coefficient based on the sun zenith angle (ϑ), fb is the beam fraction, or the percentage of direct radiation reaching the probe, and τ is the transmittance, or the ratio of below- and above-ground PAR. A is a constant coefficient that takes leaf radiation absorption into consideration. For each measurement point inside a plot, three different sub-replicates were taken, each consisting of five consecutive LAI measurements aiming at minimizing sudden canopy movements.

The concentration of chlorophyll, anthocyanin, and flavonoids was measured at the same four places in each plot by averaging readings from the top and bottom leaves using a DUALEX (Force A, Paris, France).

2.5 Destructive LAI measurements

LAI was also measured destructively by collecting half a square meter of wheat plants at point A for one replica of the damaged plots and for the control. The leaves were then separated from the stem and spikes portion, and all were placed flattened on a white homogeneous background surface Then a picture was then taken, with a Canon 1200D reflex camera with an 18-55 mm lens (Canon, Tokyo, Japan). A ruler was also placed in the scene to assure image scaling. Fiji software was used to process the images (National Institutes of Health, Bethesda, MD, US). Prior to the analysis, images were cropped and rescaled to the same resolution. Three thresholding approaches were used to separate the vegetation from the background for measuring the leaf area index: (1) a thresholding approach involving the software package's Weka Segmentation plugin. A random forest machine-learning technique underpins the plugin. The program was trained to discriminate between vegetation and background, (2) the second approach employed an automated threshold based on the Otsu algorithm, (3) the third method used a non-automatic thresholding method, where a manual threshold was used. Figures 4 to 7 provide an example of methods applied.

Figure 4: RGB image used for destructive LAI computation through image analysis

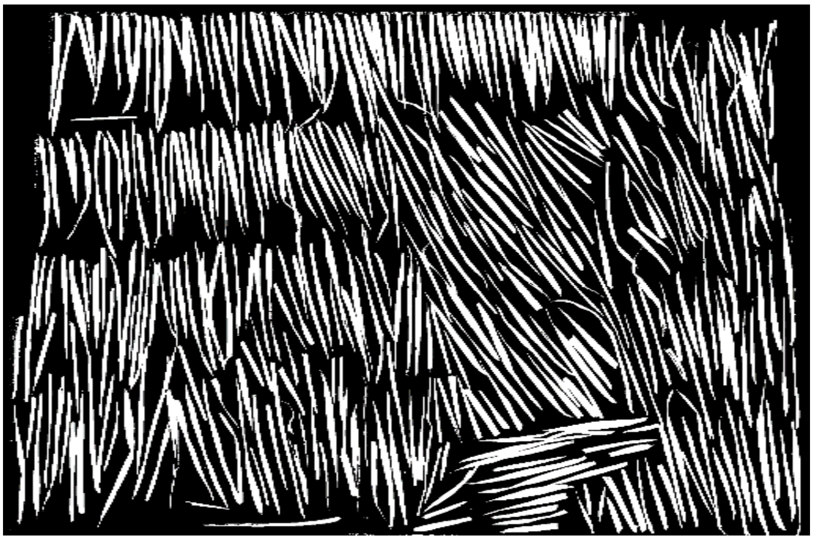

Figure 5: Thresholded 8-bit image with Otsu thresholding method

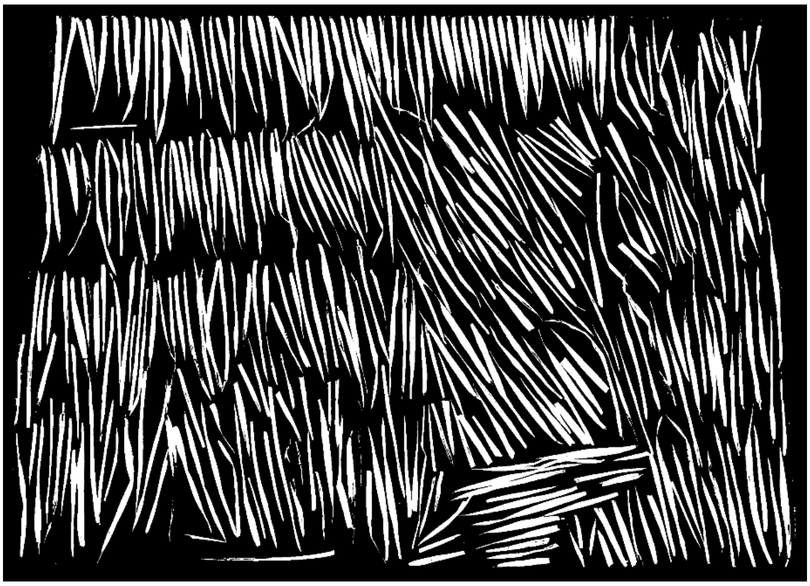

Figure 6: Thresholded image with Weka segmentation tool

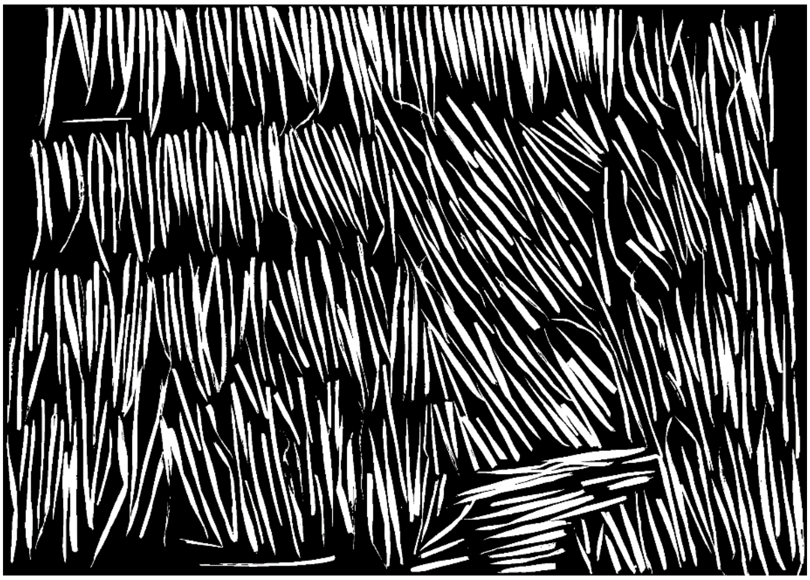

Figure 7: Thresholded image using a manual thresholding method

2.6 Statistical analysis

Statistical analysis was performed using the R software with specifically written scripts. A linear mixed-effect model with nesting was used, accounting for random errors. Treatment's damage levels (none, 20%, 50% and 80%) were used as factors to predict the investigated parameters in the model, namely LAI, chlorophyll, anthocyanins and flavonoids content and yield. The replicate and the sampling point were used to account for the random error in the nesting structure replicate/sampling point. Analysis were performed using an $\alpha = 0.05$. Anova and Tukey post-hoc comparison tests were also performed.

3 Results

3.1 Leaf Area index of wheat with destructive method

The leaf area index of wheat was assessed using the direct approach (destructive) at two developmental phases (flowering and milky) in treated and untreated plots. The first approach involved a manual image segmentation (Figure 8), that showed a clear difference in the flowering and milky stages of the crop between control and damaged plots. The control plots had LAI (6.65) at the flowering stage, while the damaged plots had a lower value. There is also a slight increase in LAI from 20% (LAI = 5.23) to 50% (LAI = 5.47) at the flowering stage, with the lowest LAI (5.14) measured at 80% damage intensity. At the milky stage, a clear decrease is evident as the damage intensity increases, with all values falling below the control and showing an average difference following the increasing defoliation level between lower damage and medium/high damages. Little difference is though observed between 50% and 80%, with only a 2.2% LAI decrease in the latter.

Fig. 9 depicts the outcome of the same destructive procedure compared with a different image thresholding methodology (Otsu method). The control plots had LAI (6.30) at the flowering stage, while the damaged plots had a lower value. The Otsu method also showed an increasing trend, the same as the manual method, with the lowest LAI (5.20) measured at 20% damage intensity. During the milky stage, the average LAI in the control plots (LAI = 4.96) was observed, while damaged plots showed a lower value. There is a decreasing trend evident as damage increased from 20% (LAI = 4.43) to 50% (LAI = 3.52) to 80% (LAI = 3.18). When using the third method (i.e., Weka Segmentation tool), similar considerations can be done as previously.

Through all the phases of crop, the control plots always showed higher LAI values than the damaged ones. On average, the three methods proved comparable in the results, leading to little differences in the LAI estimation. Taking the manual thresholding method as a reference and comparing the average LAI across all treatments, the two automated methods led to LAI values of 1.11% and 6.48% higher for Otsu and Weka segmentation tools respectively.

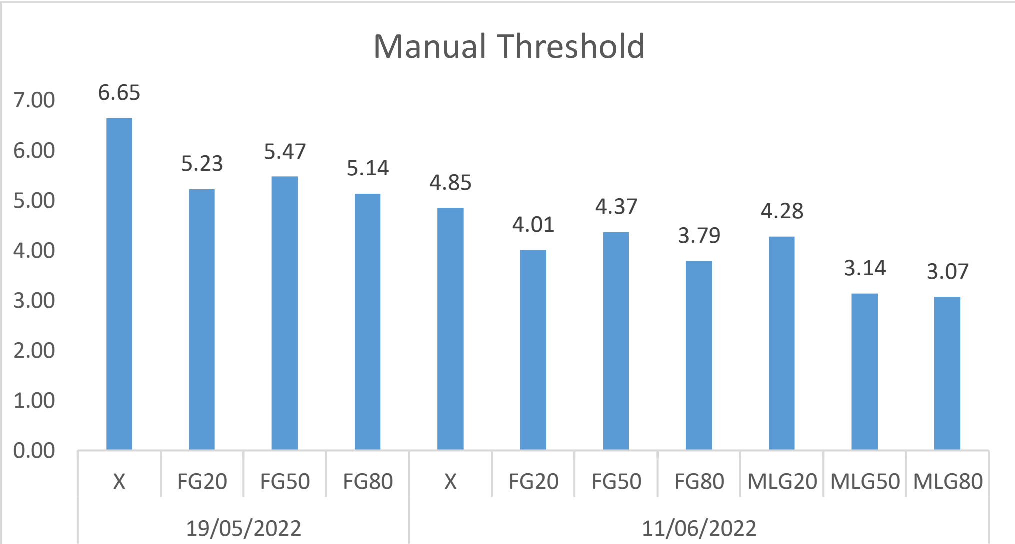

Figure 8: Leaf area index of wheat determined using the destructive approach (Manual thresholding method) during two phases (flowering, milky) in treated and untreated plots.

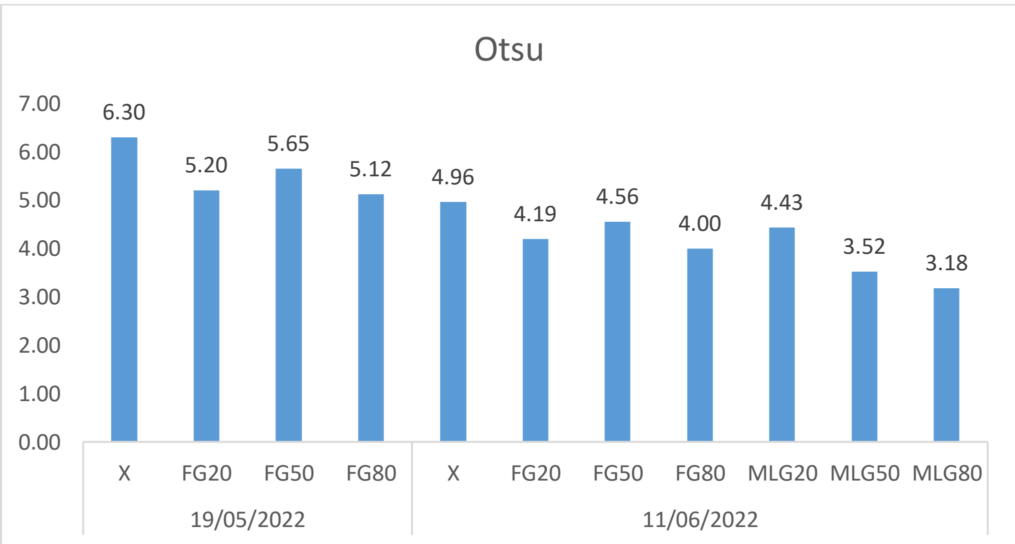

Figure 9: Leaf area index of wheat determined using the destructive approach (Otsu thresholding method) during two phases (flowering, milky) in treated and untreated plots.

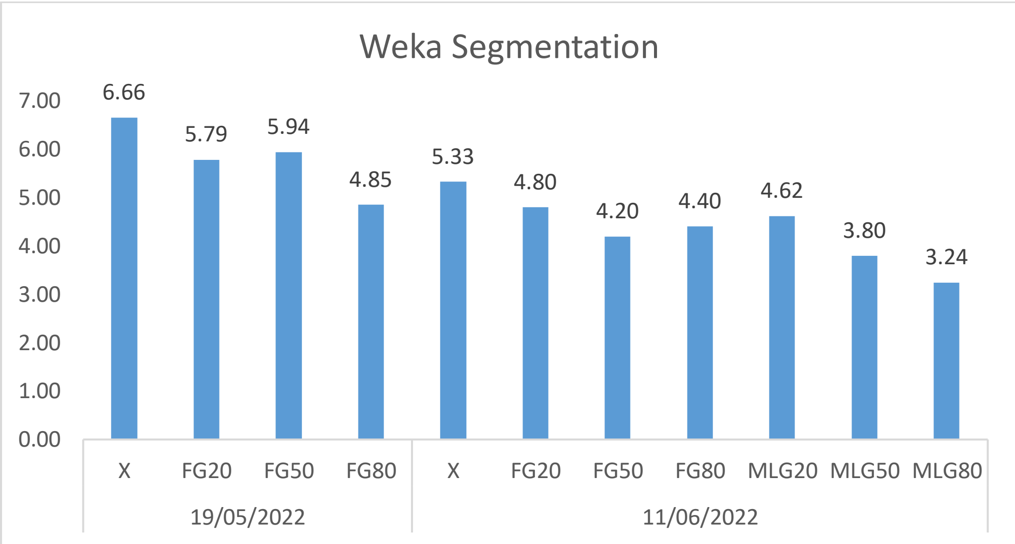

Figure 10: Leaf area index of wheat determined using the destructive approach (Weka Segmentation method) during two phases (flowering, milky) in treated and untreated plots.

The total regression for the destructive vs non-destructive leaf area index was calculated, including all treatments (Fig. 11). This figure displays a total regression between non-destructive and destructive data obtained in the lab using the manual threshold technique, with an R^2 = 0.64. A good regression was observed between the destructive and non-destructive methods when using the manual thresholding technique. In particular, good agreement was observed especially at lower and higher LAI values (points fall on the 1:1 line). More scattering was visible in the middle-range LAI values, highlighting both under and over-estimations of the non-destructive LAI compared to the destructive method.

The Otsu thresholding technique also showed good agreement at lower and higher LAI values as already observed for the manual thresholding (R^2 = 0.55). Middle range LAI values showed more scattering as well, similarly to the manual method.

The Weka segmentation tool technique proved to be the less correlated to the non-destructive LAI among the three methods (R^2 = 0.51). While similar considerations can be done for the agreement at lower and higher values, the largest scattering at middle range values was here observed. Moreover, higher LAI values are here more commonly underestimated by the non-destructive technique taking this tool as a reference.

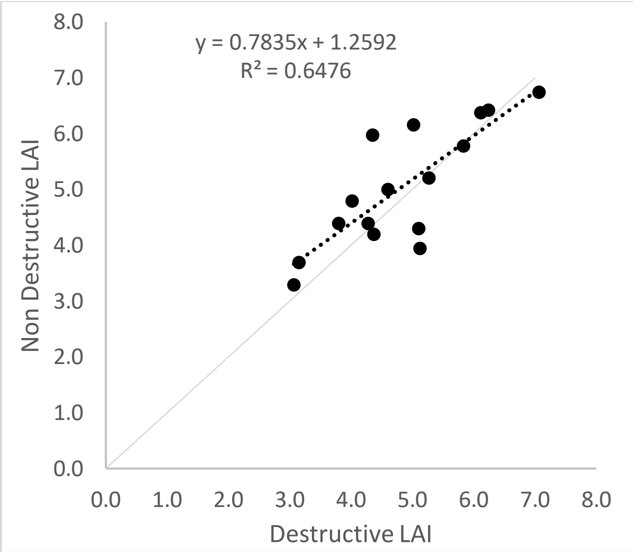

Figure 11: Destructive and non-destructive LAI of all phases of the wheat crop using FIJI software (Manual method).

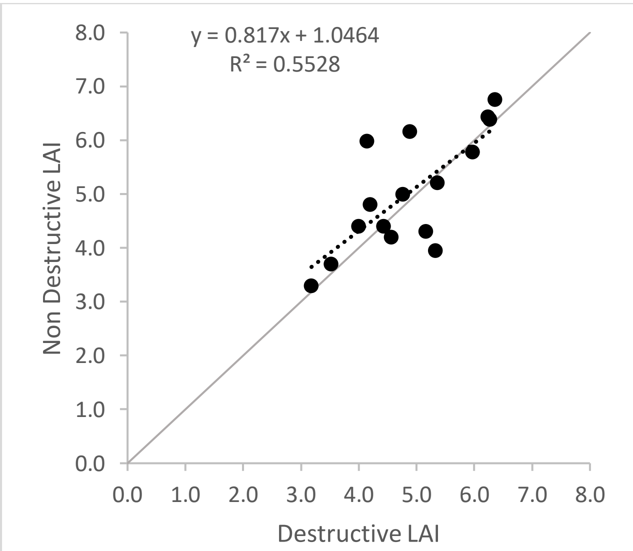

Figure 12: Destructive and non-destructive LAI of all phases of the wheat crop using FIJI software (Otsu method).

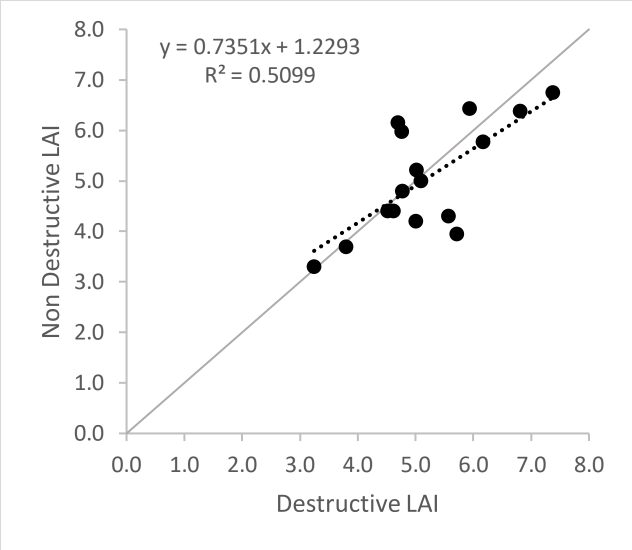

Figure 13: Destructive and non-destructive LAI of all phases of the wheat crop using FIJI software (Weka Segmentation method).

3.1.1 Leaf Area index of wheat with a non-destructive method

The wheat leaf area index was assessed after damage at the flowering stage on the 19th of May. Figure 14 shows that the average LAI value in the control treatment was 6.02±0.45, while damaged plots were 5.47±0.53, 5.29±0.43, and 5.14±0.50 (20%, 50%, and 80%, respectively). On the 14th of June, 30 days after the damage, the average LAI was 4.62±0.42 in the control plots, while the damaged plots had an average LAI of 4.46±0.46, 4.69±0.30, and 4.53±0.31 (20%, 50%, and 80%, respectively). Interestingly, little to no differentiation between treatments was here observed, particularly not between control and damaged plots. On July 1st, the control plot had an average LAI of 3.68±0.69, while the damage plots showed a decreasing trend as the percentage of damage increased (20% to 50% and then to 80%).

In the first survey of the flowering stage, control plots had greater LAI values than damaged plots. Moreover, an average decreasing trend as the percentage of damage increased is evident, following the degree of defoliation. The control plots also showed higher LAI values in the third survey than the plots damaged at 50% and 80% damage intensity. However, in the second survey similar results to damaged plots were observed, and in some cases (50%), the damaged plots had higher LAI values than control plots.

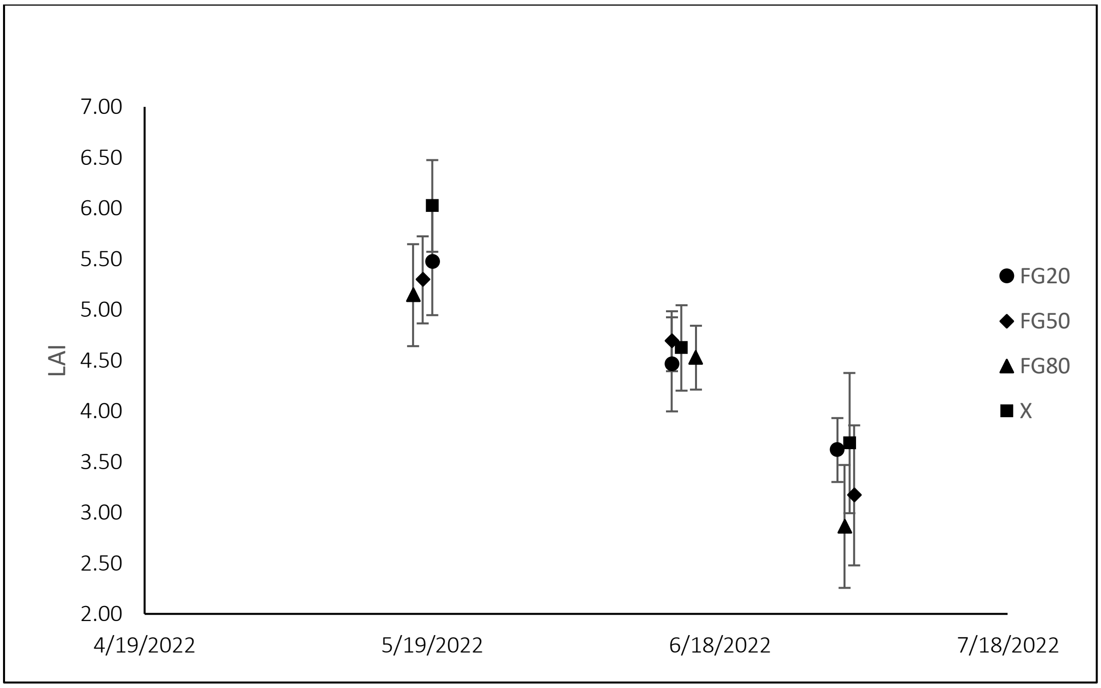

Figure 14: Leaf area index (LAI) of wheat determined using the non-destructive approach during the flowering stage.

Damages done during the milky stage were first monitored for LAI on June 14th (fig. 15). An average value of 4.62±0.42 was found in the control, whereas damaged plots revealed a LAI of 4.40±0.44, 4.11±0.12, and 4.17±0.25, (20%, 50%, and 80%, respectively). The second LAI survey was performed on July 1st and revealed an average LAI of 3.68±0.69 in the control, whereas damaged plots had LAI values of 2.99±0.31, 2.93±0.12, and 2.41±0.21 for the corresponding three increasing damage intensities.

Overall, non-damaged plots showed consistently higher LAI values across both surveys, and a slight gradient between increasing defoliation levels was also captured.

The comparison between the control and the 80% damaged plots, as well as the 50% damaged plots, revealed a significant difference on the last survey (fig. 15, $\alpha = 0.05$). When comparing 20% damaged plots to control plots, no statistical difference is though observed. Consequently, significant differences of LAI increased as the defoliation level increased.

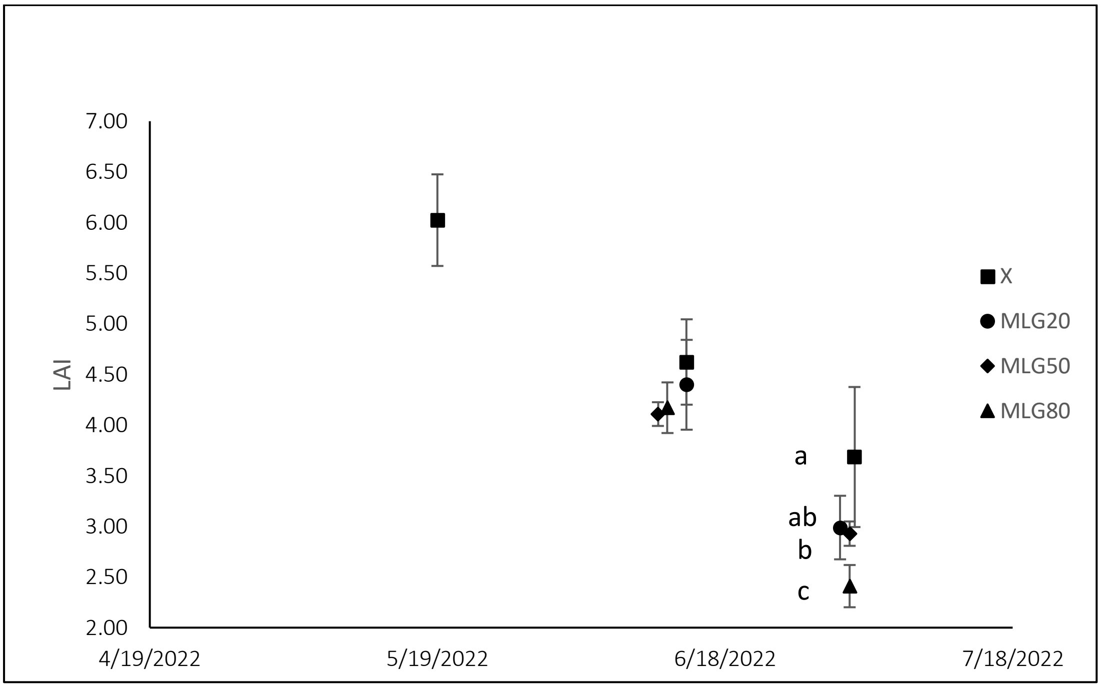

Figure 15: Leaf area index (LAI) of wheat determined using the non-destructive approach during the milky stage. Comparison letters from the post-hoc Tukey test are shown (a = 0.05).

LAI was measured once on July 1st (fig. 16) to monitor the damaged performed during the over-ripe stage. The average LAI of the control plot was 3.68±0.69, whereas damaged plots showed LAI values of 3.26±0.34, 2.73±0.11, and 2.19±0.11 (20%, 50%, and 80% respectively).

As previously observed for the other stages, the damaged plots had lower LAI values than the control plots during the crop's over-ripe stage. Furthermore, a declining trend in LAI was here seen as the damage percentage increased from 20% to 80%.A significant difference between the control plot and the plots damaged with 50% and 80% damages is evident (fig. 16, α = 0.05). No statistical difference was though observed between 20% damaged plots and control plots.

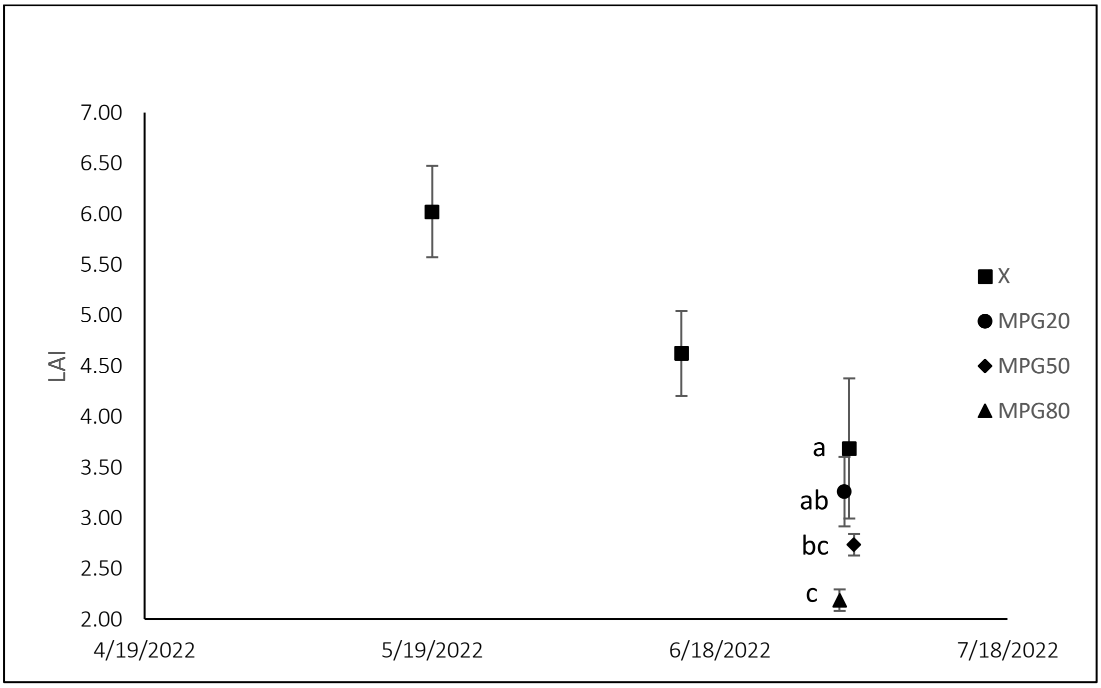

Figure 16: Leaf area index (LAI) of wheat determined using the non-destructive approach during the over-ripe stage. Comparison letters from the post-hoc Tukey test are shown (a = 0.05).

3.1.2 Chlorophyll content of wheat

On the first survey day (May 19th), the chlorophyll content was assessed during the flowering stage (Fig. 17). The average chlorophyll content of the control was 47.36±1.21 µg cm^{-2}, decreasing at 42.68±1.25 µg cm^{-2}, 40.95±1.12 µg cm^{-2} and 41.88±2.72 µg cm^{-2} in the damaged plots (20%, 50%, and 80%, respectively).

On June 14th, 30 days after the damage, the average chlorophyll content for the control plots was 35.49±1.62 µg cm^{-2}, while the damaged plots notably showed an increasing trend of chlorophyll content as the damage percentage increased (23.86±1.45 µg cm^{-2}, 26.15±1.24 µg cm^{-2}, and 27.61±4.91 µg cm^{-2}, at the three damage intensities), contrarily to what observed during the first survey. Nonetheless, across the first two survey dates the control plots always showed a higher chlorophyll content than the damaged plots.

On July 1st, the control plots had an average chlorophyll content of 13.10±0.75 µg cm^{-2}, and damage plots had chlorophyll contents of 17.65±0.60 µg cm^{-2},17.23±0.75 µg cm^{-2}, and 17.96±2.05 µg cm^{-2} (20%, 50%, and 80%, respectively). Interestingly as the severity of the damage increased (20% to 80%), so did the chlorophyll content, reversing the trend previously observed.

The chlorophyll concentration changed over time when damage occurred during the flowering stage. When compared to treated plots, control plots generally showed higher values during the first two surveys. Later in the season, at the physiological milky stage, a tendency was also evident, but it also showed an upward trend among damage treatments as the damage intensity increased. On the other hand, damaged plots had higher chlorophyll content than the undamaged ones during the last survey date.

The statistical analysis revealed a significant difference between the control and 50% and 80% damaged plots during the first surveying date. On the second survey, however, a significant difference was found between control and all other damaged plots regardless of the intensity (fig. 17).

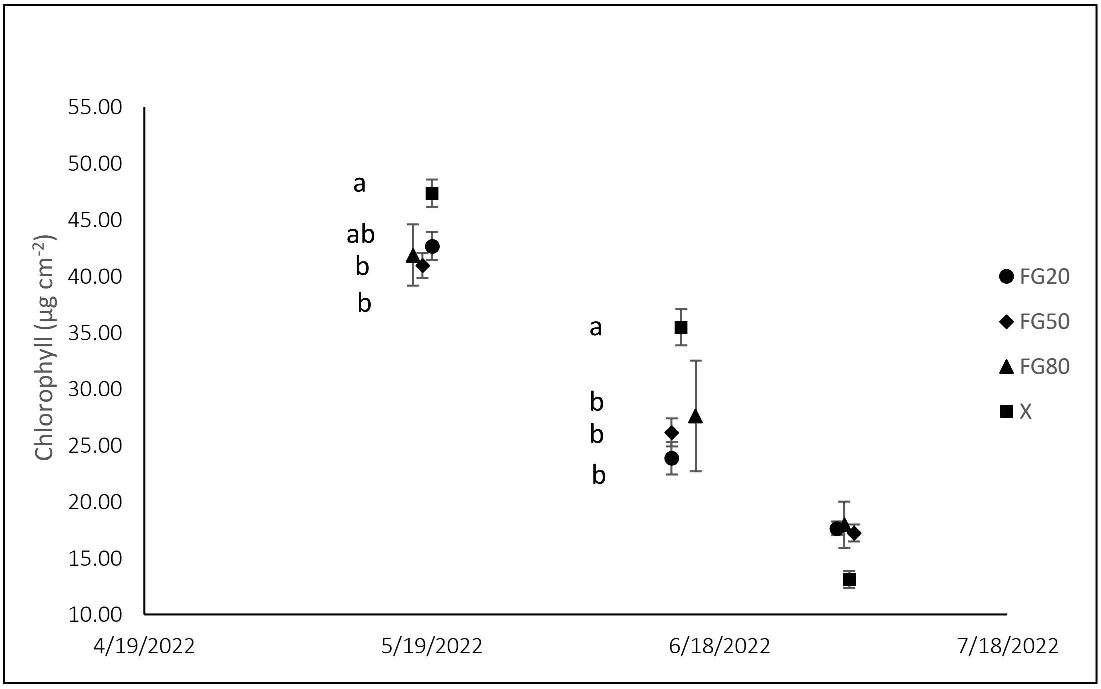

Figure 17: Changes in chlorophyll concentration of winter wheat during the flowering stage in damaged compared to undamaged wheat. Comparison letters from the post-hoc Tukey test are shown (a = 0.05).

The first chlorophyll measurements for the milky stage damaged plots were collected on June 14th (Fig. 18), and the average chlorophyll content for the control plot was 35.49±1.62 µg cm^{-2}, whereas damage plots had 29.37±1.30 µg cm^{-2}, 32.07±1.20 µg cm^{-2}, and 27.96±3.00 µg cm^{-2} (20%, 50%, and 80%, respectively).

On July 1st, the control plots had an average chlorophyll content of 13.10±0.75 µg cm^{-2}, whereas the damaged plots had 16.38±1.21 µg cm^{-2}, 17.15±1.10 µg cm^{-2}, and 16.39±1.51 µg cm^{-2} (20%, 50%, and 80%, respectively). Average chlorophyll values varied between the control plot and the 20%, 50%, and 80% damaged plots. The trend is visible in the first survey, the control plots showed higher values than the damaged plots. When plots harmed at the milky stage were surveyed again at the over-ripe stage later in the season, it was found that the damaged plots had higher chlorophyll content than untreated plots, confirming what already observed for the plots damaged at the flowering stage

Following the statistical analysis, no difference was found between control and lower damage intensity. However, a significant distinction was observed between the control and the 50% and 80% damaged plots during the first survey. All damages are though statistically equivalent.

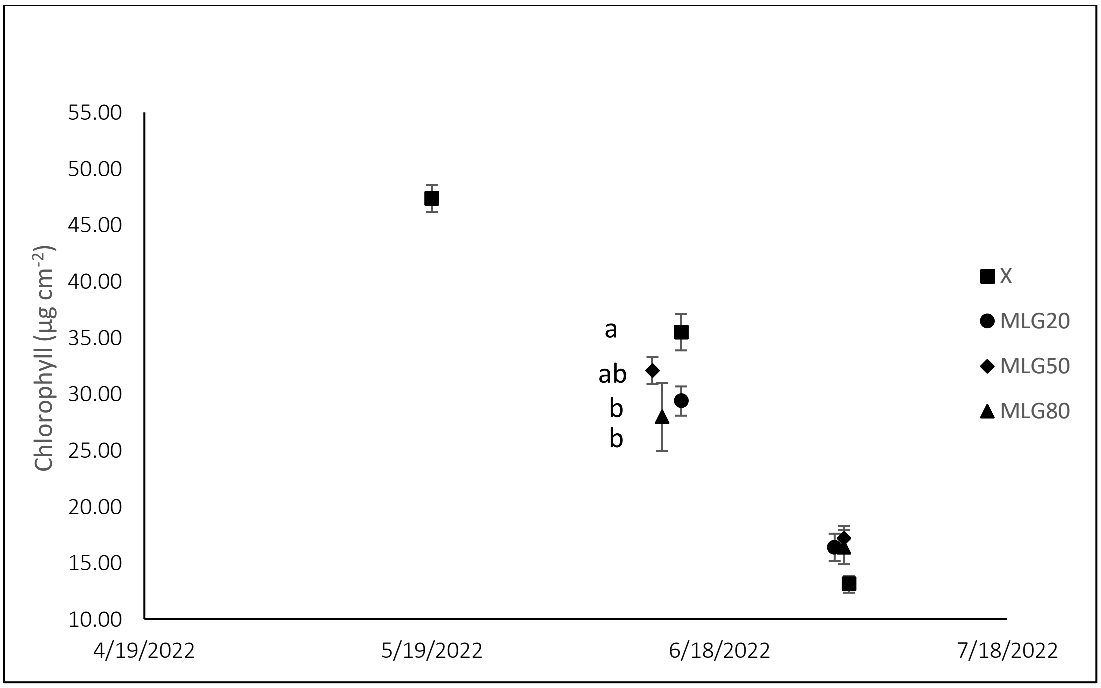

Figure 18: Changes in chlorophyll content of winter wheat during milky stage in damaged vs undamaged wheat. Comparison letters from the post-hoc Tukey test are shown (a = 0.05).

Plots damaged during the over ripe stage were measured on July 1st (fig. 19). The average chlorophyll content in control plots was 13.10±0.75 µg cm^{-2} where damaged plots it was 15.96±1.46 µg cm^{-2}, 14.71±0.89 µg cm^{-2}, and 13.38±0.67 µg cm^{-2} (20%, 50%, and 80%, respectively). Here, the previously observed upward trend on the last survey still was present, even though less pronounced than when damage occurred at flowering or milky stages.

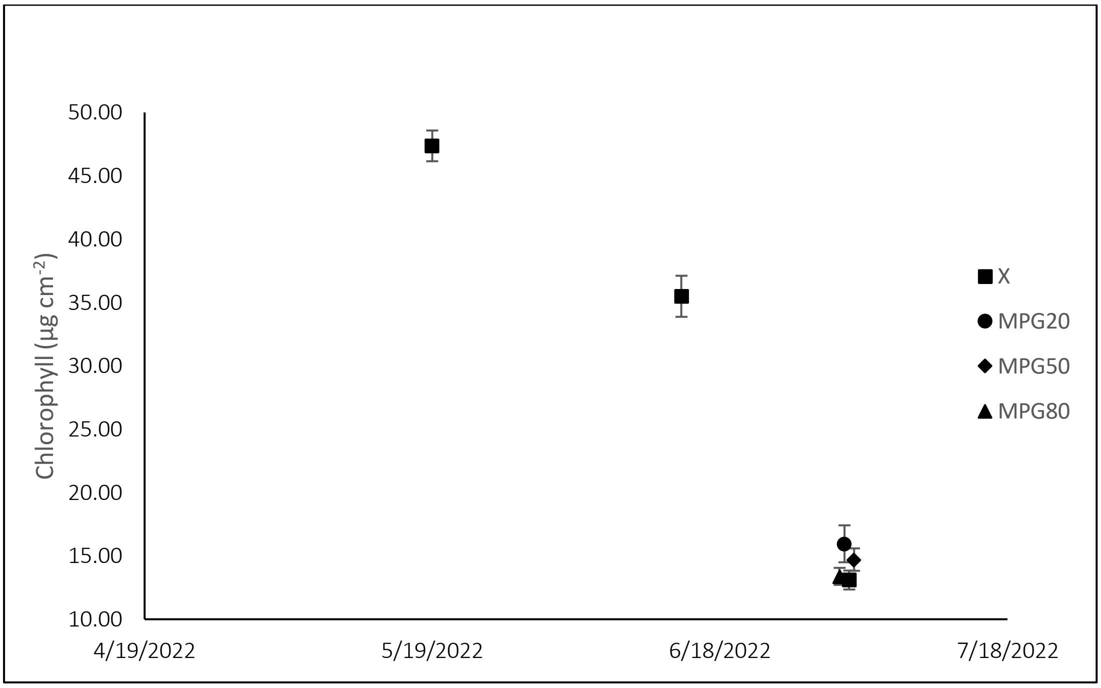

Figure 19: Changes in chlorophyll concentration of winter wheat during the over-ripe stage in damaged vs. undamaged wheat.

Defoliation damage influenced the chlorophyll content of wheat differently depending on whether it occurred during the flowering stage or during the milky and over-ripe stages. The chlorophyll content of the control plots in the flowering and milky plots was higher than that of the treated plots. The chlorophyll content of the same plots in the over-ripe stage was lower than that of the 20%, 50%, and 80% damaged plots, indicating a reaction later in the season and distinguishing the undamaged and slightly damaged plots from the higher damage intensities.

3.1.3 Anthocyanin content of wheat

Wheat anthocyanin content was assessed after damage at the flowering stage on the 19th of May (Fig. 20). The average anthocyanin content of the control was 0.12±0.01 Relative Absorbance Units (RAS), whereas values slightly increased to0.13±0.00, 0.14±0.01, and 0.14±.0.01 RAS in 20%, 50% and 80% treatments respectively.

On June 14th, about 30 days after the damage was performed, the average anthocyanin content of the control rose to 0.15±0.01 RAS, still lower than the values observed for the three defoliation levels, respectively 0.25±0.02, 0.22± 0.02, and 0.21±0.03 RAS. Interestingly, in this case a downward gradient from the top damage (80%) to the lower (20%) was present.

On July 1st, the average anthocyanin content of the control was non-detectable, whereas for the damage levels values averaged 0.02±0.02 RAS in the 20%, 0.06±0.04 RAS in the 50%, and 0.08±0.04 RAS in the 80% damage level.

The anthocyanin concentration changed over time as damage occurred during the flowering stage. When compared to control plots, damaged plots always showed higher anthocyanin values. A trend was also seen when plots harmed during the flowering stage were examined later in the season at the physiological milky stage.

ON the last survey, however, anthocyanin content was nom-detectable in the control plots, whereas the damaged plots showed a consistent increasing trend as the defoliation level increased from 20% to 80%.

No significant difference was found between 20% and 50% damaged plots compared to control plots. However, there is a significant difference between the control plots and the 80% damaged plots. All damages were among them statistically similar.

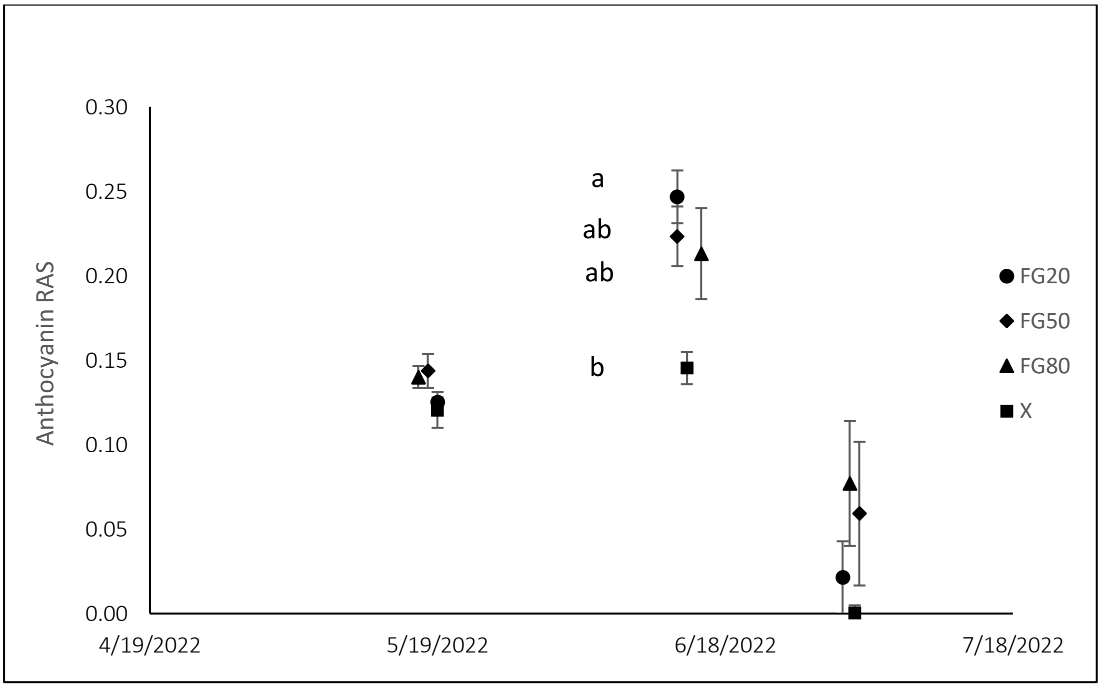

Figure 20: Changes in anthocyanin concentration of winter wheat during the flowering stage in damaged vs. undamaged wheat. Comparison letters from the post-hoc Tukey test are shown (a = 0.05).

On June 14th, the first survey following damage at the milky stage was carried out (fig. 21). The control plots showed an average anthocyanin content of 0.15±0.01 RAS, whereas the damage levels showed higher values, i.e., 0.18±0.01, 0.19±0.01, and 0.19±0.02 (20%, 50% and 80% respectively).

On July 1st, the average anthocyanin content of the control non-detectable, whereas the damage levels averaged 0.22±0.02, 0.05±0.01, and 0.02±0.06 RAS (20%, 50% and 80% respectively). Notably, 20% defoliation level showed considerably higher values than other treatments.

The average anthocyanin values differed between the control plot and the 20%, 50%, and 80% damaged plots. The trend is clear in the first survey, when damage simulation occurred at the milky stage, the control plots had lower values than the damaged plots. When plots harmed during the milky stage were examined later in the season at the over-ripe stage, anthocyanin content was not detected in the control plots, and it also showed a downward trend as the damage intensity increased.

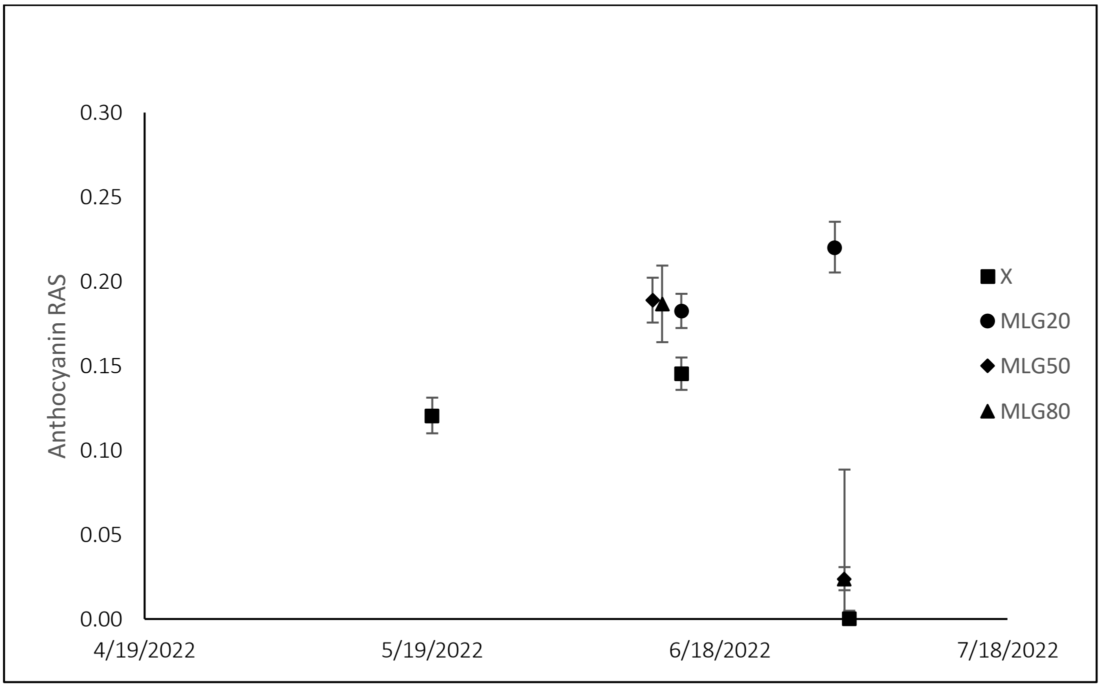

Figure 21: Changes in anthocyanin concentration of winter wheat during the milky stage in damaged vs. undamaged wheat.

On July 1st, the survey was conducted during the over-ripe stage of wheat (fig. 22). The average anthocyanin content of the control was non-detected, whereas for the damage treatments was 0.09±0.00 RAS, 0.06±0.00 RAS, and 0.01±0.00 RAS (20%, 50% and 80% respectively).

Hail damage seemed to affect the anthocyanin content of wheat differently depending on whether it occurred during the flowering, milky, and over-ripe stages. The anthocyanin content of the control plots was lower than that of the treated plots in the flowering stage, and it was not detected in the milky and over-ripe stages, and damaged plots showed a decreasing pattern as the percentage of damage increased.

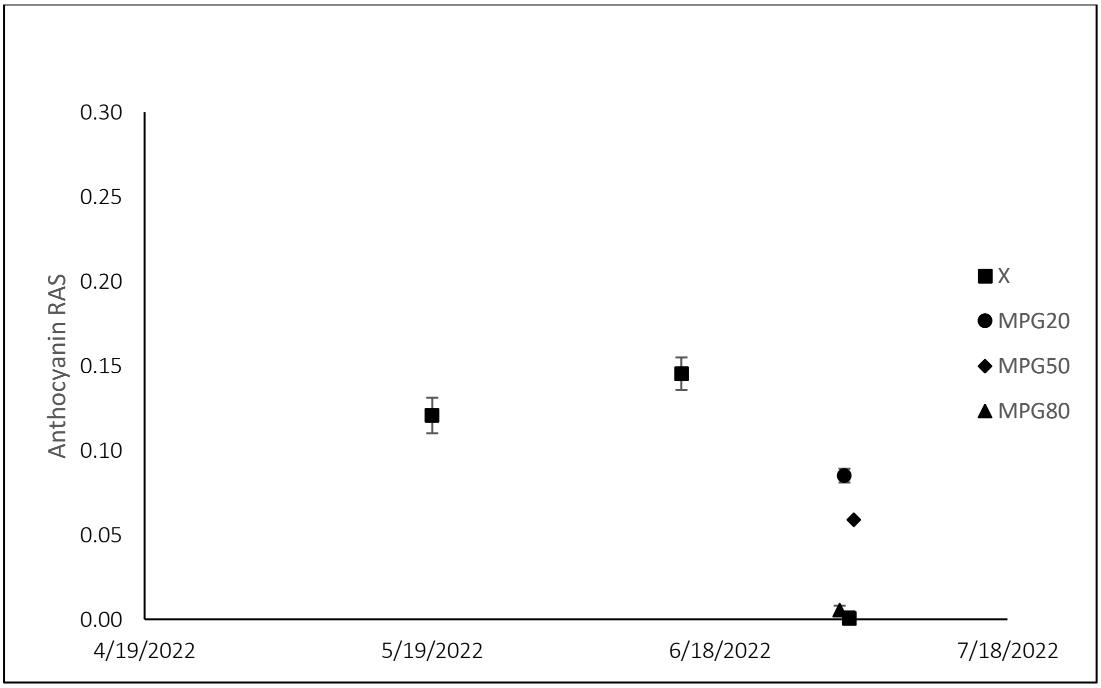

Figure 22: Changes in anthocyanin concentration of winter wheat during the over-ripe stage in damaged vs. undamaged wheat.

3.1.4 Flavonoids content of wheat

Wheat flavonoids concentration was assessed following damage at the flowering stage on the 19th of May (Fig. 23). The control plots average flavonoids content was 1.38±0.03 RAS, rising in the damage levels to 1.45±0.01, 1.48±0.01, and 1.49±0.04 RAS (20%, 50% and 80% respectively).

Similarly, on June 14th, around 30 days after the damage assessment was done, the control plots average flavonoid content was 1.48±0.02 RAS. The damage treatments still averaged higher values compared to the untreated plots that is 1.66±0.03, 1.58±0.05, and 1.56±0.04 RAS (20%, 50% and 80% respectively).

During the last survey on July 1st, the control plots average flavonoid content dropped at 0.03±0.00 RAS, whereas in the damage treatments was 0.06±0.04, 0.18±0.10, and 0.32±0.26 RAS for 20%, 50% and 80% respectively).

The flavonoids' concentration changed over time as damage simulation occurred during the flowering stage. When compared to control plots, damaged plots had generally higher flavonoid content. A trend was also seen when plots harmed during the flowering stage were examined later in the season at the physiological milky stage, where control plots showed lower values than the damaged, but also a downward trend as the damage intensity increased was observed, reversing previous survey's observations. On the last survey date, the flavonoid content of control plots was still lower than that of damaged plots, whereas damaged plots showed an increasing trend as the percentage increased from 20% to 80%, confirming the previous survey gradient.

The statistical analysis revealed a significant difference between the control and 50% and 80% damaged plots on the first survey date. The same plots, however, were analyzed again at the milky stages, and no difference between the control and 20% and 50% damaged plots was observed. Still, a significant difference was still found between the control and 80% damaged plots.

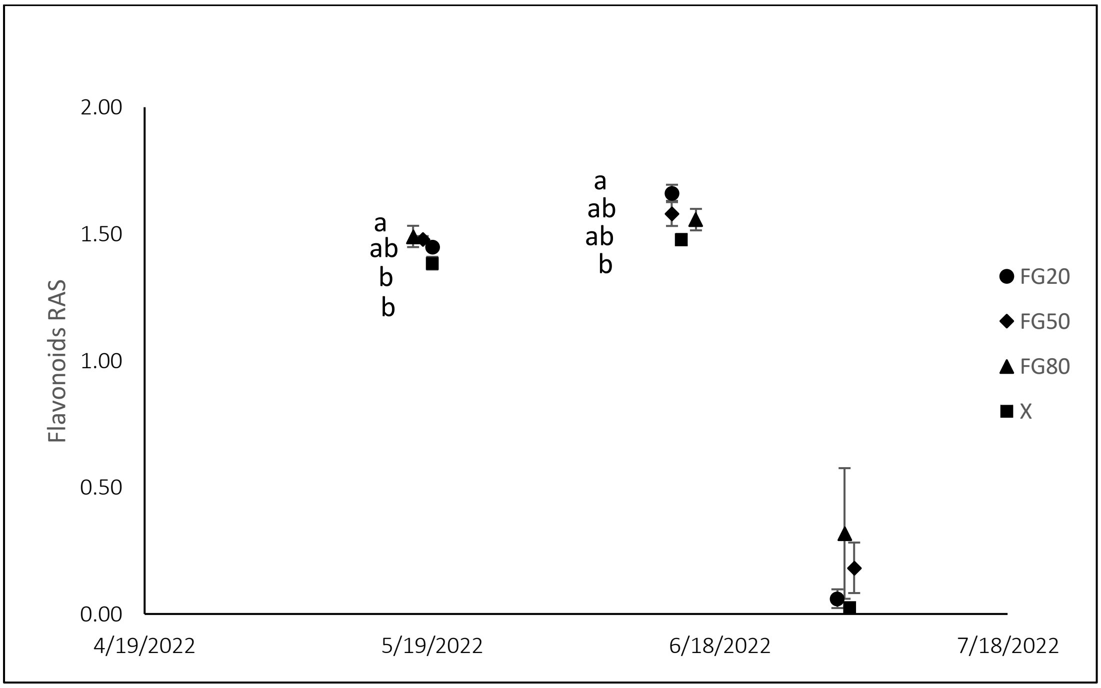

Figure 23: Comparison of flavonoids concentrations in damaged vs. undamaged winter wheat at the flowering stage. Comparison letters from the post-hoc Tukey test are shown (a = 0.05).

On June 14th, the first survey was performed on the damaged milky stage plots (fig. 24). The average flavonoid content of the control plots was here 1.48±0.02 RAS, slightly higher than the lower damage (1.47±0.03 RAS) but lower when compared to the higher defoliation levels (1.53±0.02 RAS and 1.53±0.03 RAS).

On July 1st, the average flavonoid content in the control plots was 0.03±0.00 RAS, considerably lower compared to the average values observed in damage treatments, that is 0.33±0.07, 0.21±0.38, and 0.54±0.21 RAS for 20%, 50% and 80% respectively.

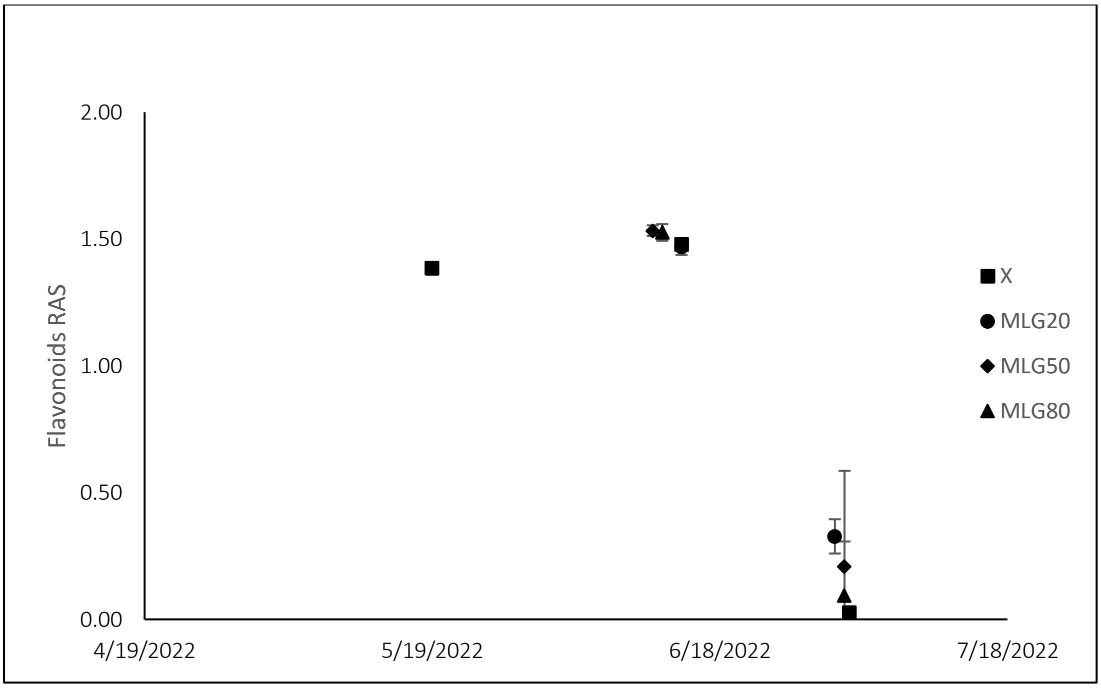

Figure 24: Comparison of flavonoids concentrations in milky stage of winter wheat from damaged and undamaged plants.

On July 1st, a survey was done to monitor the plots damaged during the over-ripe stage (fig 25). The control plots had an average flavonoid content of 0.03±0.00 RAS, notably lower compared to the 20% damage level values of 0.35±0.05 RAS. Interestingly, 50% and 80% defoliation levels had distinctively lower average values, equal to 0.06±0.03 RAS, and 0.09±0.06 RAS respectively.

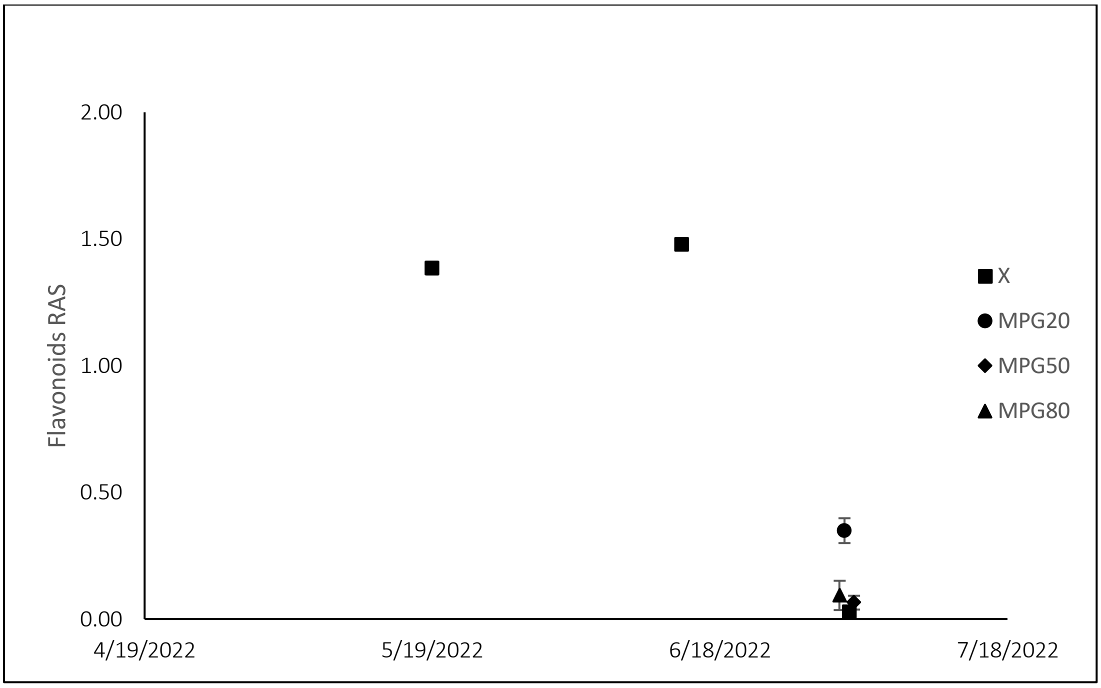

Figure 25: Comparison of flavonoids concentrations in damaged vs. undamaged winter wheat at the over-ripe stage.

3.2 Yield analysis

When compared to the treated plots, the yield of the control plots showed the highest values, averaging at 7.44 t ha^{-1} (fig. 26). The lowest values were always observed when the higher levels of defoliation occurred, averaging 3.37 t ha^{-1}, 3.99 t ha^{-1} and 2.67 t ha^{-1} in flowering, milky and over-ripe stages respectively. Generally, as the defoliation level increased at all different growth phases of the plant, the yield decreased accordingly, highlighting a positive correlation between yield decrease and simulated hail damage intensity increase.

The statistical analysis revealed a significant difference between the control and all other damaged plots in all the stages of crop growth. During the flowering stage, the control plots showed an average yield of 7.44 tons/ha, while the damaged plots showed 4.11, 3.78, and 3.36 tons/ha. Little differentiation was generally found between 20% and 50% damage over all stages. At the milky and over-ripe stages, similar patterns were observed. During the over-ripe stage, there was a little difference observed between the 20% (5.06 tons/ha) and 50% (4.72

tons/ha) damage, but when compared with the 80% (2.67 tons/ha), there was a greater difference. The largest reduction in the yield occurred when the crop was damaged at high intensities in all growing stages.

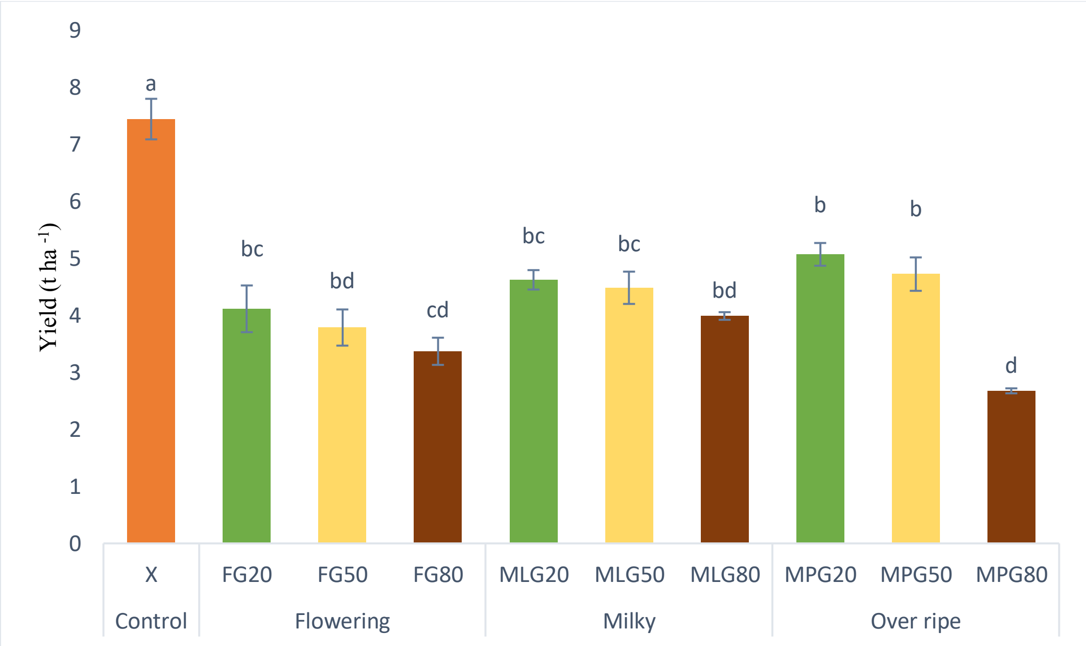

Figure 26: Obtained yield following damage at varying degrees of severity during the different periods of crop development. Comparison letters from the post-hoc Tukey test are shown (a = 0.05).

4 Discussion

LAI was measured at various growing stages of the plant, that is flowering, milky, and over-ripe stages. Different LAI analysis techniques were used, that is destructive and non-destructive methods. Given the handiness and quick estimation provided by the non-destructive LAI estimation, a comparison between the latter and the destructive method was carried to assess the reliability of the less invasive technique. The ceptometer values provided a reliable estimate of LAI in wheat, particularly when compared against the destructive manual thresholding technique, where the two methods had a good overall agreement. Points were mainly positioned on the 1:1 line, and the correlation coefficient was equal to 0.65, confirming the usefulness and reliability of the indirect measurements taken with a ceptometer. that is based on a modified version of the canopy light transmission and scattering model(Campbell, 2020). LAI measured during the cropping season using the indirect method showed a more efficient, less time-consuming, and less labor-consuming method for monitoring the vegetation. This considered, LAI values measured with the ceptometer gave a good estimate of LAI in winter wheat, showing on average a good agreement with the destructive method. According to Blanco and Folegatti, leaf area is one of the important parameters for understanding photosynthesis, light interception, water and nutrient use, and crop growth. It is thus critical to accurately measure the leaf area of the crop in order to gain a better understanding of the relationships between crop development and the environment. The direct method is more precise, but it is also more time- and labor-intensive. However, the non-destructive methods (regression techniques) based on linear measurements of plant leaves are relatively accurate, quicker, and effortless to implement in several crops (Singh et al., 2018). Wheat chlorophyll content was evaluated in treated and untreated plots. During the flowering and milky stages of wheat, control plots had an averagely higher chlorophyll content than damage treatments, while during the over-ripe stage damaged plots had higher values than the control, suggesting a specific plant response to damage in later stages. As reported in the literature, hail damage alters the structure of crop canopies and alters light absorption thus affecting the distribution of the pigments, such as chlorophyll (Bijanzadeh & Emam, 2010). In the case of wheat, when an increase of chlorophyll was observed in later stages, this might be the case in which plants respond to damage later in the season taking advantage of the increased amount of light reaching the middle and bottom part of the canopy following defoliation. The anthocyanin content of wheat throughout all phases of crop growth showed that the damaged plots had an average higher anthocyanin content than the control plots. A similar outcome was observed in grapes. Defoliation causes an increase in grape anthocyanin content due to sunlight-driven stimulation of polyphenol production. Indeed, the control had the lowest anthocyanin accumulation compared to hail damaged treatments, and this was most likely due to the higher sun exposure of the latter (Ćirković et al., 2022). Wheat crop flavonoids content showed that the damaged plots had a higher flavonoids content than the control plots during all growth stages of crop, similar to what was observed for anthocyanin. In the grapevine, a similar result was observed (Ćirković et al., 2022). For example, for plants such as grapevines, the variability in the concentration of phenolic acids and flavonoids in the evaluated wines was strongly impacted by defoliation. Wheat hail damaged plots resulted in lower yields than control plots. The yield decreased as the proportion of damage increased at all different stages of the plant. When the crop was injured at greater intensities throughout the growing season, the production dropped the most. During the milky stage of wheat, stem fracture prevents nutrients from traveling to the spikes, decreasing the total volume of grain weight and kernel weights, and hence the yield, although stem breaking reduces the number of kernels per spike during other stages, such as the over-ripe period (Busch, R. H., 1968). According to Ahmadi and Joudi (2007), defoliation may have no effect on yield since the loss will be compensated by an increase in net photosynthetic rate and a rise in the stability of net leaf chlorophyll (Busch, R. H., 1968). Breaking of stems, particularly spikes, can therefore result in yield losses (Ferguson et al., 1987).

5 Conclusion

This study assessed how hailstorms affected several vegetative features and variables in winter wheat. The research revealed that when compared to the control, the damaged plots exhibited a distinct effect in the leaf area index, and in chlorophyll, anthocyanins and flavonoids content. This difference seems to be based on the stage of plant growth at which the defoliation occurs. Different LAI measurement techniques were tested at several phases of the plant, such as flowering, milky, and over-ripe stages. The indirect (non-destructive) and direct (destructive) LAI measurements showed a good agreement, indicating the reliability of LAI indirect measures using a ceptometer even in non-standard canopy structures as when defoliation occurs.

A clear average downward trend proportional to the damage intensity in the yields was also observed in treated plots among them and when compared to control treatment. When the crop was treated with the highest intensity of defoliation, the yield was reduced the most. More research is although needed on how the plant responds to a mix of external stresses such as the combination of hailstorms and, e.g., pathogen attacks, as well as on how these results extend to other developmental stages of winter wheat.

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