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Short-term (during minutes) changes in the composition of weed leaves exposed to abiotic stress

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ABSTRACT

Exposure to abiotic stress can lead to various physiological, biochemical, and molecular changes in plants. In 2025, a field experiment was conducted to study the rapid (within the first 15-30 minutes) response of two weeds, dandelion and couch grass, to mechanical damage (sequential cutting of leaf pieces). Raman spectroscopy was used to analyze the plant samples. The focus was on organic compounds such as aliphatic compounds, aromatic rings, carotenoids, pectin, phenylpropanoids, and starch. Several other organic compounds and complexes have also been identified that could potentially play an important role in plant resistance to stress factors. The concentration of different organic compounds in control and damaged plant leaves varied with time. These changes differed in the morning and afternoon. This might be caused by differences in external environmental factors and also short-term circadian variations of the plant's physiological activity. The temporal fluctuations of most organic compounds in the leaves of dandelion and couch grass were different. Furthermore, the plants responded differently to abiotic stress. This may be due to the fact that couch grass is a monocot, while dandelion is a dicot. The physiological and biochemical processes in these plants can differ significantly. The response of plants to leaf cutting was very rapid. The concentration of organic compounds could vary up to three times compared to the control. However, in many cases, plants recovered from mechanical impact within a short period of time, 30 minutes.

Contribution/Originality: The paper's primary contribution is finding that concentrations of various organic compounds in plants can fluctuate over a short period. Rapid plant responses to mechanical stress can be significant and differ between monocots and dicots. In many cases, plants are able to recover fairly quickly after mechanical impact.

1. INTRODUCTION

Plants are often exposed to various negative environmental conditions. The impact of abiotic stress factors on plants leads to extremely complex physiological, biochemical, and molecular changes [1, 2]. However, higher plants have a unique ability to resist these adverse effects. It is important to understand the ability of different plant species to adapt to such stressful conditions. Despite numerous attempts during the last decades to investigate the processes resulting from plant injury and some progress that was made towards the solution of this problem, there is still insufficient research into the effect of mechanical damage on different organic compounds, especially those with significant antioxidant activity [3]. Until now, mechanical damage, particularly that caused by cutting off a part of a plant leaf, has been studied much less compared to other abiotic and biotic environmental stress factors [4].

Meanwhile, such stressful situations often occur in nature. It is known that leaf cutting can trigger a cascade of various biochemical changes [5]. These changes may vary among plant species and may result in either a decrease or an increase in the concentration of certain organic compounds [6, 7].

This complex task is complicated by the fact that all living organisms, including plants, are not static systems; their physiological state is constantly changing. Among others, circadian fluctuations contribute significantly to these changes. The internal biological clock is a central feature of all living organisms. It enables plants to keep track of time, allowing them to adapt their physiological processes and thus optimally respond to the rotation of our planet [8, 9]. Circadian rhythms in plants allow them to anticipate daily changes in the environment. This helps plants respond to various biotic and abiotic stresses [10]. Circadian rhythms are constantly adjusted under the influence of external environmental factors [11]. In addition, plant composition can be influenced by a wide range of external natural factors, such as temperature and humidity fluctuations, the geomagnetic field, ozone levels in ground-level air, and many others.

In our study, weeds such as couch grass and dandelion were used to examine the effects of mechanical damage on plant leaf metabolism. Compared to agricultural crops, the biochemical composition of weeds is not analyzed as often. Meanwhile, weeds can become a useful tool for predicting the impact of various factors on plants.

In the work, an attempt was undertaken to understand the role of aliphatic compounds, aromatic rings, carotenoids, pectin, phenylpropanoids, and starch in their immediate response, within the first 15-30 minutes, to abiotic stress. These organic compounds are essential molecules that perform a wide variety of functions in plants. Among them, carotenoids are very important for various biological processes in plants; they are essential for photosynthesis and photoprotection. Carotenoids serve as signaling molecules that respond to environmental changes [12] and have been shown to be reliable indicators of plant response to stress factors [13, 14]. Carotenoids vary by plant species; any change in carotenoids caused by environmental changes is specific to that species.

Selecting a suitable instrumental analytical method to determine the effect of mechanical stress on organic compounds in plants is a challenging task. In our case, Raman spectroscopy was used to analyze plant samples. This method is considered one of the most promising non-destructive techniques for determining various compounds in plants. It allows rapid measurement of molecular structures without pre-treatment of samples [15, 16]. Raman spectroscopy has also been successfully used to diagnose early plant responses to abiotic stress [17, 18].

The main objectives of this study were to investigate the inhibitory and/or enhancing effects of abiotic stress (cutting off a part of a plant leaf) on the accumulation of selected organic compounds in plant leaves and to understand the mechanisms of these effects. Another objective was to study the influence of short-term natural fluctuations and climatic conditions on the behavior patterns of organic compounds in two weed species belonging to different classes: monocots and dicots.

2. MATERIALS AND METHODS

2.1. Sampling Site and Protocol

The experimental site was located 96 km north of St. Petersburg, Russia (60°42′59″N, 30°03′24″E). The site measured approximately 2.5 x 2.5 m. On October 18, 2025, leaves of two common weeds, couch grass (*Elytrigia repens*) and dandelion (*Taraxacum officinale*), were collected multiple times at 15-minute intervals in the morning (from 8:30) and mid-afternoon (from 13:00). Leaves of control plants (growing separately) and leaves of plants after sequential cutting of leaf fragments were collected simultaneously. The scheme of the experiment (on the example of dandelion) is shown in Figure 1. A completely randomized design was used for sampling. Each time, at least three plant samples were collected. The soil characteristics at the site are presented in Table 1. Data on external factors on the day of sampling are also shown in Table 2.

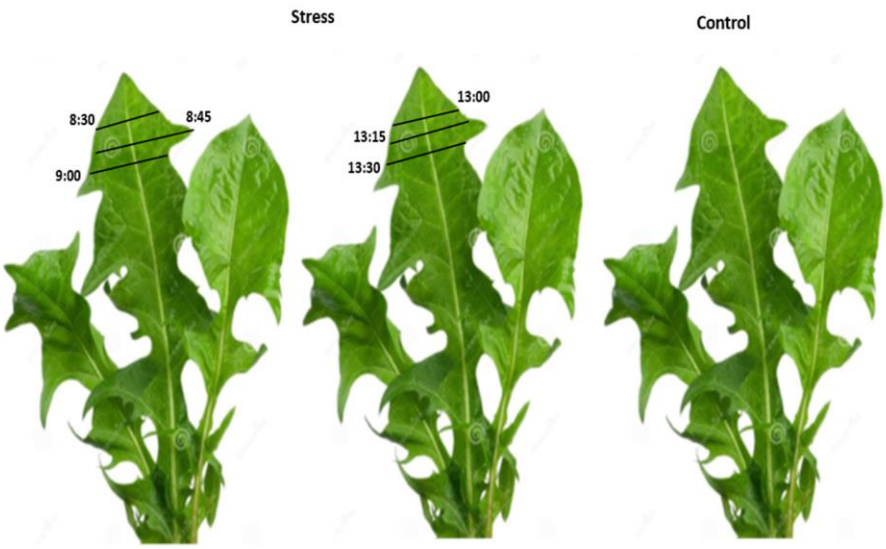

Figure 1. Scheme of experiment.

Table 1. Parameters of bulk soil.

Texture	Sand	Silt	Clay	Total carbon	pH
sandy loam	59.47%	39.25%	1.28%	5.75%	7.55

Table 2. Characteristics of the experimental site on the day of plant sampling.

Time of sampling	Temperature, °C	Humidity, %	Nitric oxide, mg/m³	Ozone, mg/m³	Atmospheric pressure, mmHg	MF, Kp
8:30	0.3	71	0.270	0.024	764.2	2.7
8:45	0.3	70	0.287	0.021	764.3	2.8
9:00	0.4	71	0.229	0.018	764.5	2.9
13:00	7.7	60	0.077	0.030	765.3	2.0
13:15	8.3	56	0.060	0.037	765.2	1.8
13:30	8.8	54	0.040	0.042	765.2	1.7

2.2. Analysis of Soil

Soil pH (1:2.5 H_2O) was determined. The particle size distribution of soil samples was analyzed using a small-angle laser diffraction on a Shimadzu SALD-2201 laser granulometer (Japan). WingSALD software was used to process the data.

2.3. Raman Spectroscopy of Plant Samples

Raman spectra were obtained using an ORTES-785SLRam confocal Raman spectrometer. It was based on the Wasatch Photonics (USA) WP-785-R-ER-LMMFC-S-25 modular OEM spectrometer with a volume phase holographic grating (VPH) coupled with a 785 nm laser with an adjustable power of 5–450 mW. The 785 nm laser was selected to minimize luminescence from the test object, which is typically abundant in many organic substances. A Hamamatsu S16011 CCD (charge-coupled device), cooled to +10°C, was used as a photodetector, ensuring a high signal-to-noise ratio. The ORTES-785SLRam spectral range was 250–3500 cm^{-1}, with a spectral resolution of 6–8 cm^{-1}. The exposure time for spectrum acquisition was set in the range from 0.1 to 300 seconds. The number of measurements of each spectrum varied from 100 to 3000. The Savitsky-Golay algorithm was used to smooth the contour of the Raman spectrum. Spectrum preprocessing was performed using the iterative polynomial approximation baseline correction method, optimized to compensate for diffuse fluorescence bands. Further processing of the spectra obtained from the spectrometer was performed using the «OPTEC-Mobile Raman System» software package [RPO Registry No. 13217 (April 11, 2022)]. Raman spectra of plant leaves showed vibrational bands that can be assigned to starch (480 cm^{-1}), aromatic rings (668 cm^{-1}), pectin (747 cm^{-1}), aliphatic compounds

(1284 cm^{-1}), carotenoids (1525 cm^{-1}), and phenylpropanoids (1606-1632 cm^{-1}). A detailed description of measurement conditions and instrument parameters is given in our previous publication [19].

2.4. Data Analysis

Multivariate statistical analysis of the experimental data was performed using Statistica for Windows 8.0 (StatSoft, Tulsa, OK, USA). Raman band intensity data for selected organic compounds were expressed as mean values. Analysis of variance was used to assess statistically significant differences between data in different groups of samples. The significance level was set at $P<0.05$. Cluster analysis (CA) and principal component analysis (PCA) were used to examine similarities and differences between control and damaged couch grass and dandelion samples collected at different times. For CA, Ward's method was applied, using squared Euclidean distances as a similarity measure. Data for CA and PCA were normalized to prevent classification errors caused by variables with different orders of magnitude.

3. RESULTS AND DISCUSSION

3.1. Short-term Variations of Organic Compounds in Leaves of Control Plants

Figure 2 shows Raman spectra of leaves of dandelion and couch grass collected in the morning (at 8:30) and in the middle of the day (at 13:00). Raman scattering intensity (RSI) of different compounds in leaves of control (undamaged) plants changed over several hours. In the dandelion, the RSI of all selected organic compounds increased from morning to midday. In couch grass, the temporal changes of the RSI of different organic compounds also varied. The RSI of aliphatic compounds, carotenoids, and phenylpropanoids decreased significantly from morning to afternoon. The RSI of pectin increased slightly, while the RSI of starch and aromatic rings remained almost unchanged. As seen from Figure 2, significant differences were found in the RSI of the same organic compounds in dandelion and couch grass growing in the same place and collected at the same time. Moreover, the temporal fluctuations in the RSI of most organic compounds in the leaves of dandelion and couch grass were different. For example, in dandelion leaves, the RSI of a particular compound could increase from morning to midday, while in couch grass leaves, it could decrease significantly. One possible reason for these changes may be circadian fluctuations, which may vary among different plant species.

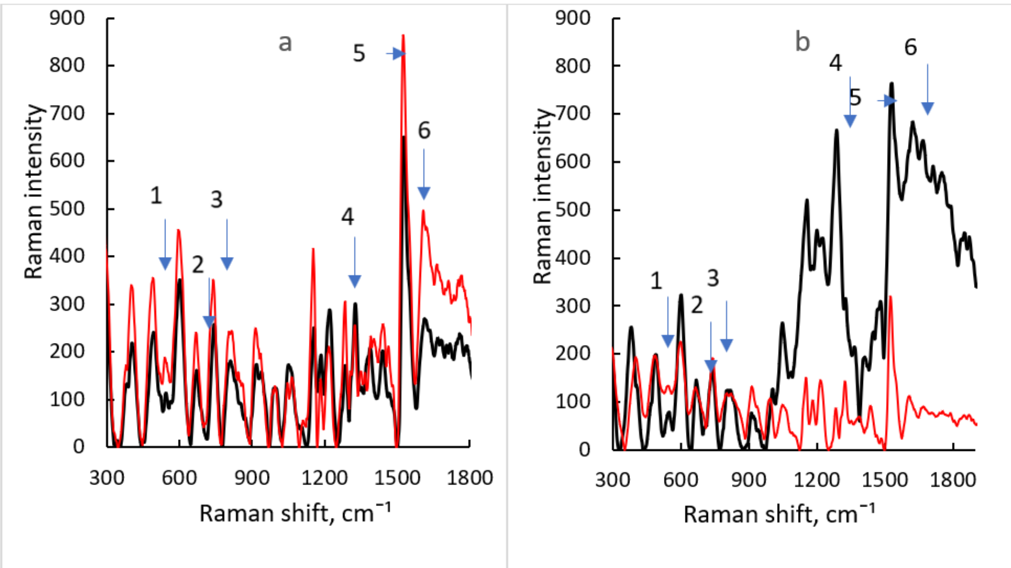

Figure 2. Raman spectra of leaves of control plants collected in the morning (Black) and afternoon (Red). a – dandelion, b – couch grass. 1 – starch, 2 – aromatic rings, 3 – pectin, 4 – aliphatic compounds, 5 – carotenoids, 6 – phenylpropanoids.

Another factor capable of affecting short-term changes in plant composition may be varying environmental conditions. As the data in Table 2 suggest, from morning until midday, air temperature increased, and humidity decreased. The NO concentration decreased significantly, while O_3 increased. This relationship between NO and O_3 was expected, as NO levels increase in the morning and O_3 levels rise with increasing temperature and solar radiation [20]. The effects of NO and O_3 on plants are well known and described in the literature [21-25]. Therefore, variations in NO and O_3 concentrations in ground-level air could also affect plant chemical composition.

It has been reported that natural geomagnetic storms correlate with changes in the biological processes of living organisms [26]. The different effects of magnetic fields (MF) on plants may depend on the plant species, as well as the exposure time and MF intensity [27]. As can be seen from Table 2, in the morning, there was some increase in the MF. By 13:00, the level of geomagnetic disturbances returned to a calm state. It should be noted, however, that published results of experiments studying the influence of weak magnetic fluctuations on living organisms often contain contradictory data [28]. On the one hand, the mitigating effects of MF on plants in response to abiotic and biotic stresses have been reported [29, 30]. But on the other hand, weak MF can cause certain adverse effects [31, 32]. It is also important to note that changes in the geomagnetic field likely influence plant circadian cycles [33].

The results obtained indicate that many possible factors can influence plants by changing the concentration of various organic compounds in leaves over a relatively short period. Numerous external and internal factors affecting plant life can interact with each other. It can be assumed that it is hardly possible to consider the influence of only one factor, regardless of the others.

3.2. The Plant's Reaction in the First Minutes after Cutting off a Piece of Leaf

Figure 3 shows the spectra of dandelion leaves (control plants and plants after sequential leaf cutting) collected every 15 minutes in the morning. The first (at 8:30) mechanical damage resulted in an increase in the RSI of aliphatic compounds and carotenoids and a decrease in pectin and phenylpropanoids. Additionally, a significant increase in the RSI was detected at peaks of 1196 cm^{-1} and 1408 cm^{-1}. These peaks in the Raman spectra of plants may be associated with secondary metabolites formed as a result of stress, when plants under stressful conditions increase the production of certain defense compounds [34]. These compounds help plants adapt to unfavorable environmental conditions. In response to abiotic stress, including mechanical damage to plant leaves, plants activate complex signaling and effector systems. In these systems, not only ion homeostasis but also the dynamic formation of labile biomolecular complexes plays a key role [35].

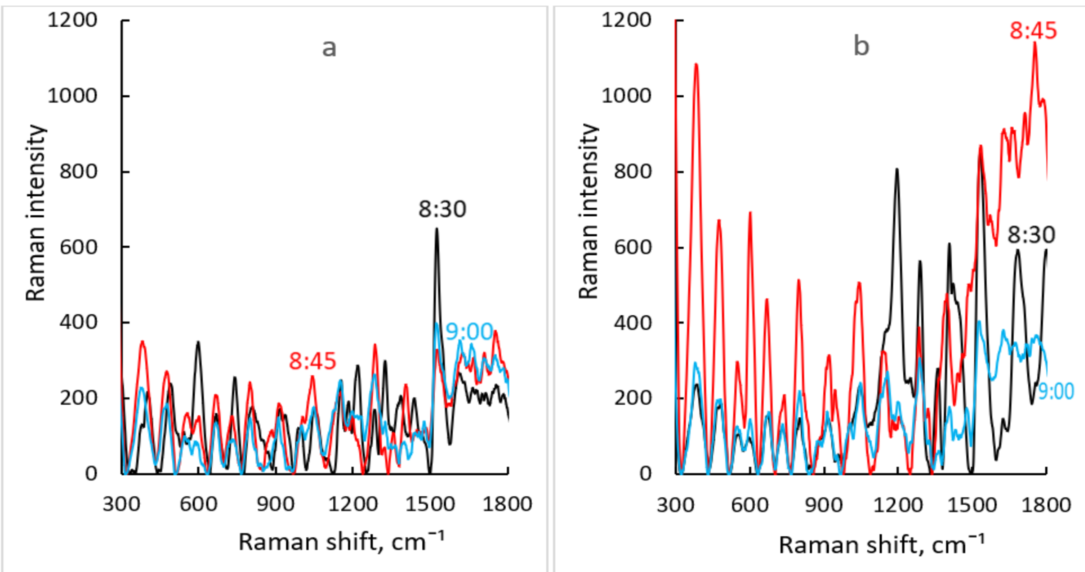

Figure 3. Raman spectra of leaves of dandelion collected in the morning. a – control, b – after the plant leaf has been cut several times.

After another 15 minutes (at 8:45), the RSI of aliphatic compounds in the next piece cut from the leaf decreased. The RSI of starch, aromatic rings, pectin, and phenylpropanoids increased. The RSI of carotenoids did not change over this period (Figure 3b). After another 15 minutes (at 9:00), the RSI of almost all organic compounds decreased in both cut and control plants. In leaves of mechanically damaged plants, the RSI of these substances became either almost the same or only slightly higher than in leaves of control plants. Interestingly, this happened after a fairly short period of time (30 minutes) after the first cut. Similar unstable behavior of substances after mechanical damage to *Melaleuca alternifolia* leaves was reported earlier [7]. The authors found that mechanical injury resulted in a decrease in oil concentration in *M. alternifolia* leaves within the first 24 hours, but the leaves then recovered most of the lost oil within 48 hours of injury. Our experiment showed that although mechanically induced stress can affect the concentration of some organic compounds in leaves, it does not cause pronounced changes in plant metabolism. Moreover, it looks like plants are able to acclimate to certain stress conditions and return to their original state during a rather short time. This is likely due to the fact that, in some cases, mechanical stimuli can improve plant defense responses and increase their resistance to stress [36]. Figure 4 shows changes in the RSI of carotenoids, aliphatic compounds, starch, phenylpropanoids, aromatic rings, and pectin in control and damaged dandelion leaves collected in the morning. In control plant leaves, within 30 minutes, the RSI of pectin and carotenoids decreased, phenylpropanoids increased slightly, while the RSI of other organic compounds reached a maximum at 8:45.

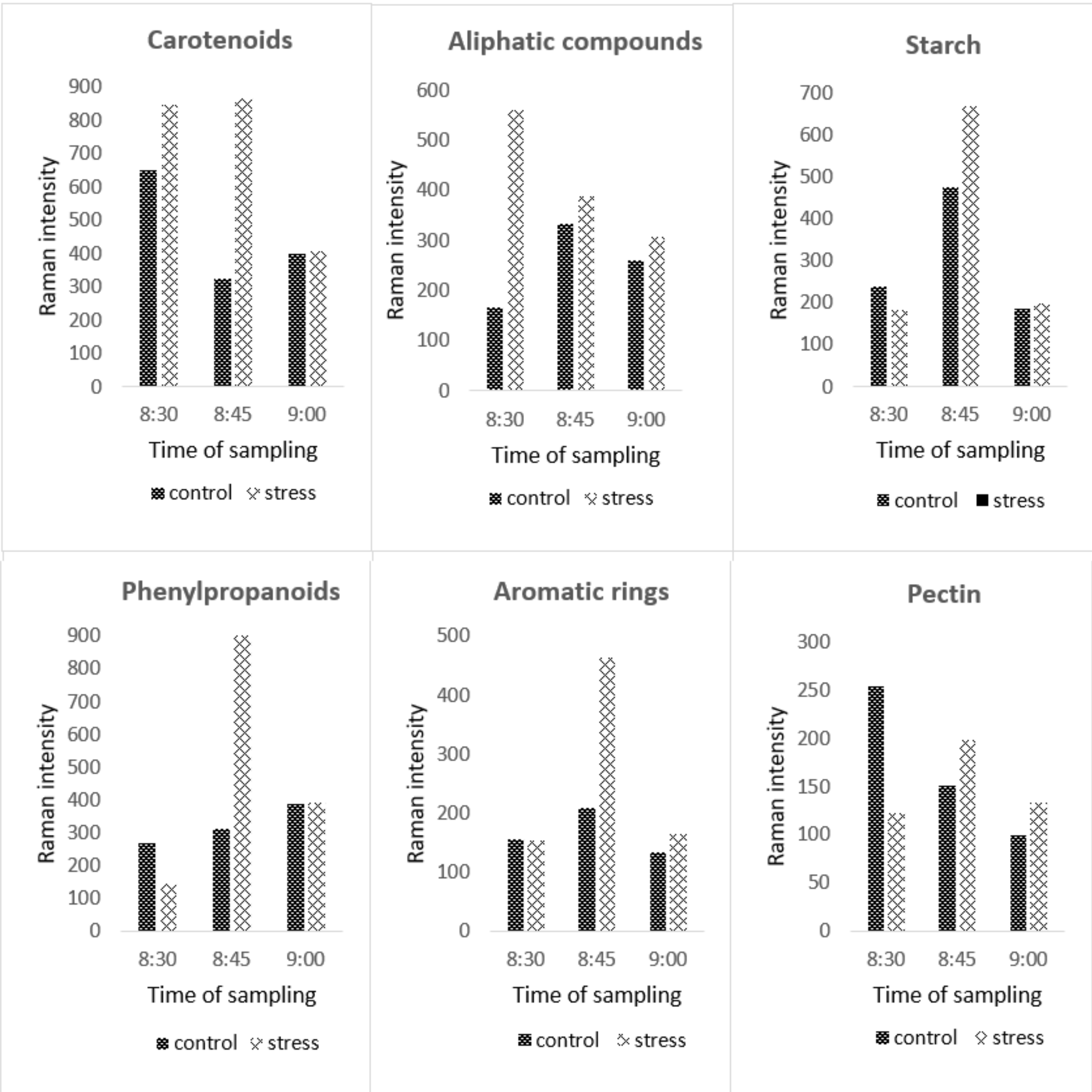

Figure 4. Changes of organic compounds in the leaves of dandelion (control and after cutting the plant leaf).

The first response of plants to stress was a significant increase in the RSI of carotenoids and aliphatic compounds and a decrease in phenylpropanoids and pectin. This reaction was observed immediately after cutting the plant leaf at 8:30. When the next piece of leaf was cut 15 minutes later, the difference between the leaves of control (undamaged) plants and the leaves of damaged plants increased. The RSI of all organic compounds increased significantly. After 15 minutes, at 9:00, when the next piece of leaf was cut, the RSI of the different organic compounds became almost the same or only slightly higher in leaves of damaged plants than in leaves of control plants. This means that it took a short time to recover plant physiological processes after mechanical stress.

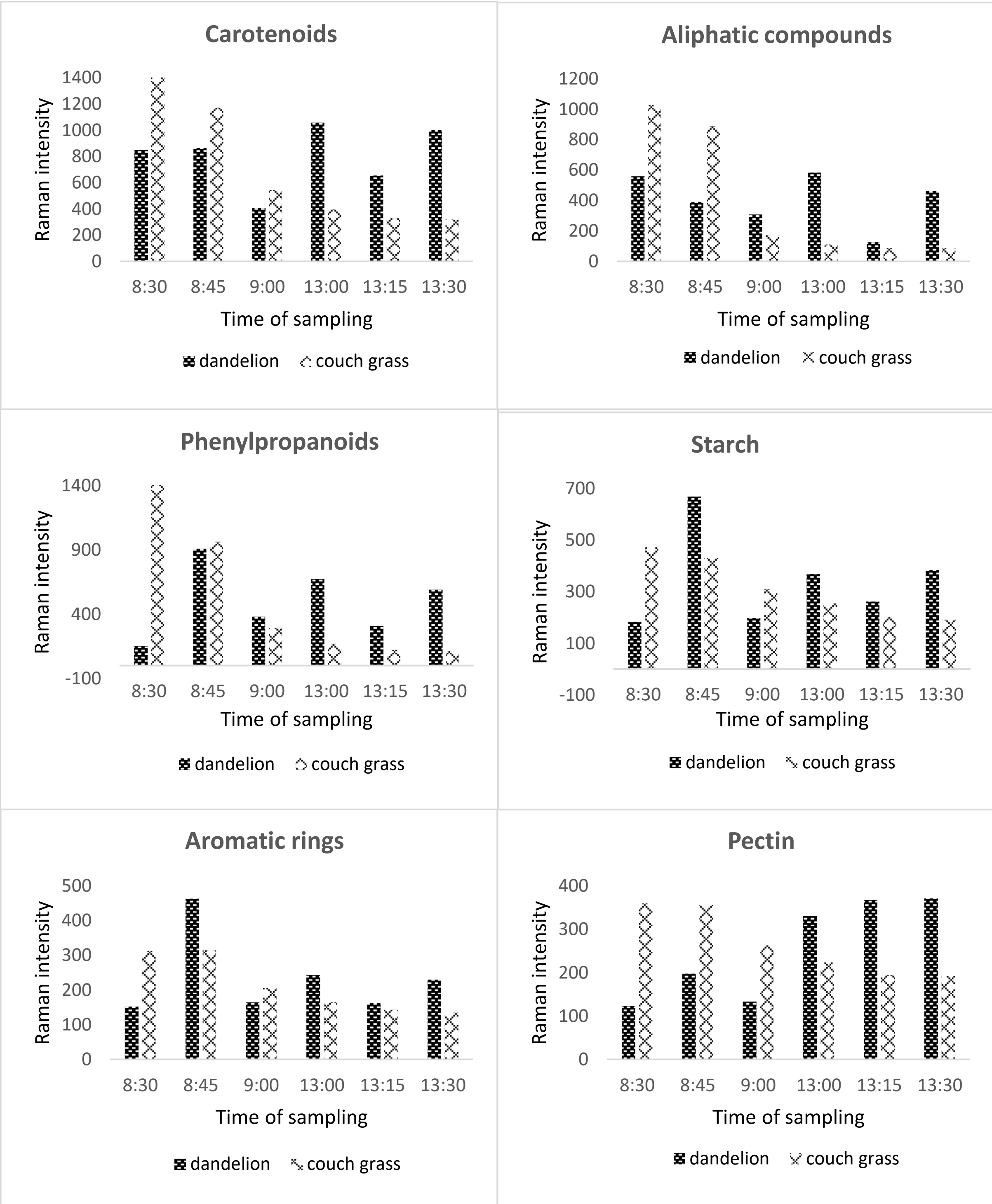

Figure 5. Raman scattering intensities of carotenoids, aliphatic compounds, phenylpropanoids, starch, aromatic rings, and pectin in leaves of damaged dandelion and couch grass collected in the morning and afternoon.

In Figure 5, the RSI of carotenoids, aliphatic compounds, phenylpropanoids, starch, aromatic rings, and pectin in leaves of damaged dandelion and couch grass are shown, collected in the morning and afternoon. Although both plants grew in the same small plot and under identical conditions, they responded differently to stress. It is noteworthy to observe the differences in plant responses to stress at different times of the day. In the morning, the RSI of organic compounds in damaged leaves of dandelion and couch grass varied significantly, often in different directions. In the afternoon, after cutting a new piece of dandelion leaf, the concentration of various organic compounds in this leaf fragment changed more over a short period than in the damaged couch grass leaf. It can be assumed that these plants have different mechanisms to counteract stress effects, and probably, at midday, couch grass was more resistant to mechanical stress compared to dandelion.

Plants are often exposed to multiple abiotic stress factors. This can lead to various physiological, biochemical, and molecular changes, which can sometimes cause a wide variety of reactions [37]. The results of our experiment indicate short-term fluctuations in the RSI of all studied organic compounds, as well as a time-dependent response of plants to mechanical stress. It can be assumed that, in addition to mechanical damage, circadian fluctuations, changes in air temperature, the concentration of NO and O_3 in ground-level air, and MF in the area, may serve as additional stimuli for such variations.

3.3. Multivariate Statistical Analysis of Experimental Data

To study the similarities and differences between couch grass and dandelion leaves (control and damaged plants), as well as to evaluate the rapid response of plants to mechanical damage and the differences in their stress responses, cluster analysis (CA) and principal component analysis (PCA) were conducted based on six components: aliphatic compounds, aromatic rings, carotenoids, pectin, phenylpropanoids, and starch.

The results of CA are presented in Figure 6. Couch grass leaves (control and damaged plants) collected at 8:30 were close to each other, slightly farther from the leaf pieces cut 15 minutes later, and well separated from the leaves cut another 15 minutes later, at 9:00. Dandelion leaves collected in the morning at the same time were separated differently.

In the afternoon, leaves of control couch grass and dandelion were separated from leaves of the plants, where leaf pieces were sequentially cut. It is also interesting that the linkage distance of the couch grass and dandelion samples collected in the afternoon differed. It was smaller for dandelion than for couch grass. From this, it is likely that in the afternoon, dandelion samples were more similar to each other than couch grass samples.

In the second stage, PCA was carried out on dandelion and couch grass leaves that were sequentially cut over a short period in the morning and afternoon. Principal component analysis showed good separation of dandelion leaves into two distinct groups, samples collected in the morning and at midday (Figure 7a). The first principal component (PC), accounting for 52% of the total variance, was responsible for the separation, and pectin was highly correlated with this PC. Couch grass leaves collected in the morning and afternoon were separated from each other by the second PC (it accounted for 19% of the total variance), with aromatic rings, starch, phenylpropanoids, and aliphatic compounds making the main contribution to this separation (Figure 7b).

According to the data presented in Figure 5, compared with other organic compounds, the RSI of pectin in dandelion leaves collected in the morning was indeed lower than in dandelion leaves collected in the afternoon. The RSI of all organic compounds in leaves of couch grass collected in the morning was higher than in couch grass leaves cut at midday.

Considering that couch grass is a monocot and dandelion is a dicot, and many physiological and biochemical processes in these plants differ, the PCA data support the hypothesis of differences in the concentration of some organic compounds between couch grass and dandelion. This may, at least in part, indicate a specific role of these compounds in plant biological processes.

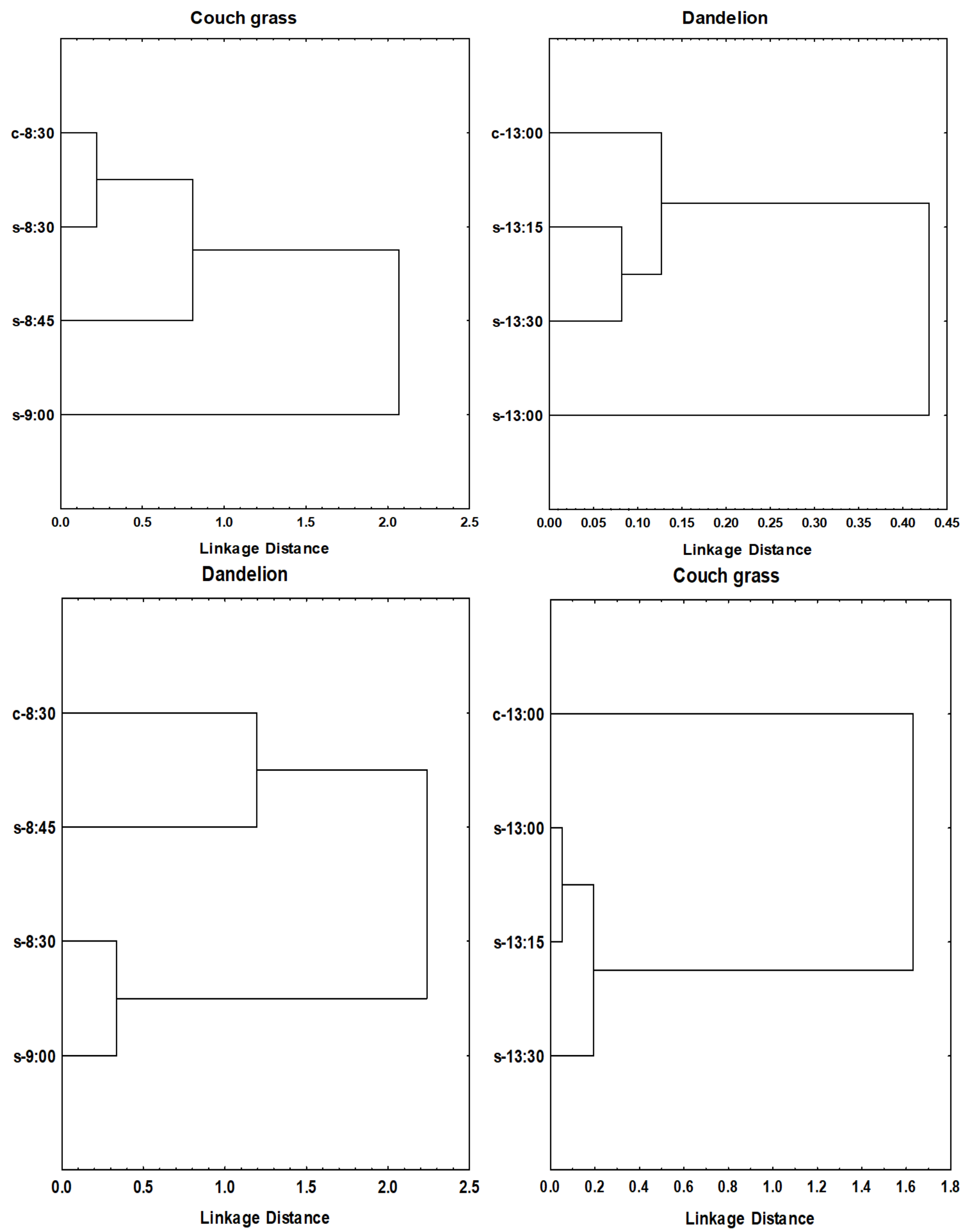

Figure 6. Cluster analysis (Ward's method) of leaves of couch grass and dandelion collected in the morning and afternoon. c – control samples, s – damaged samples.

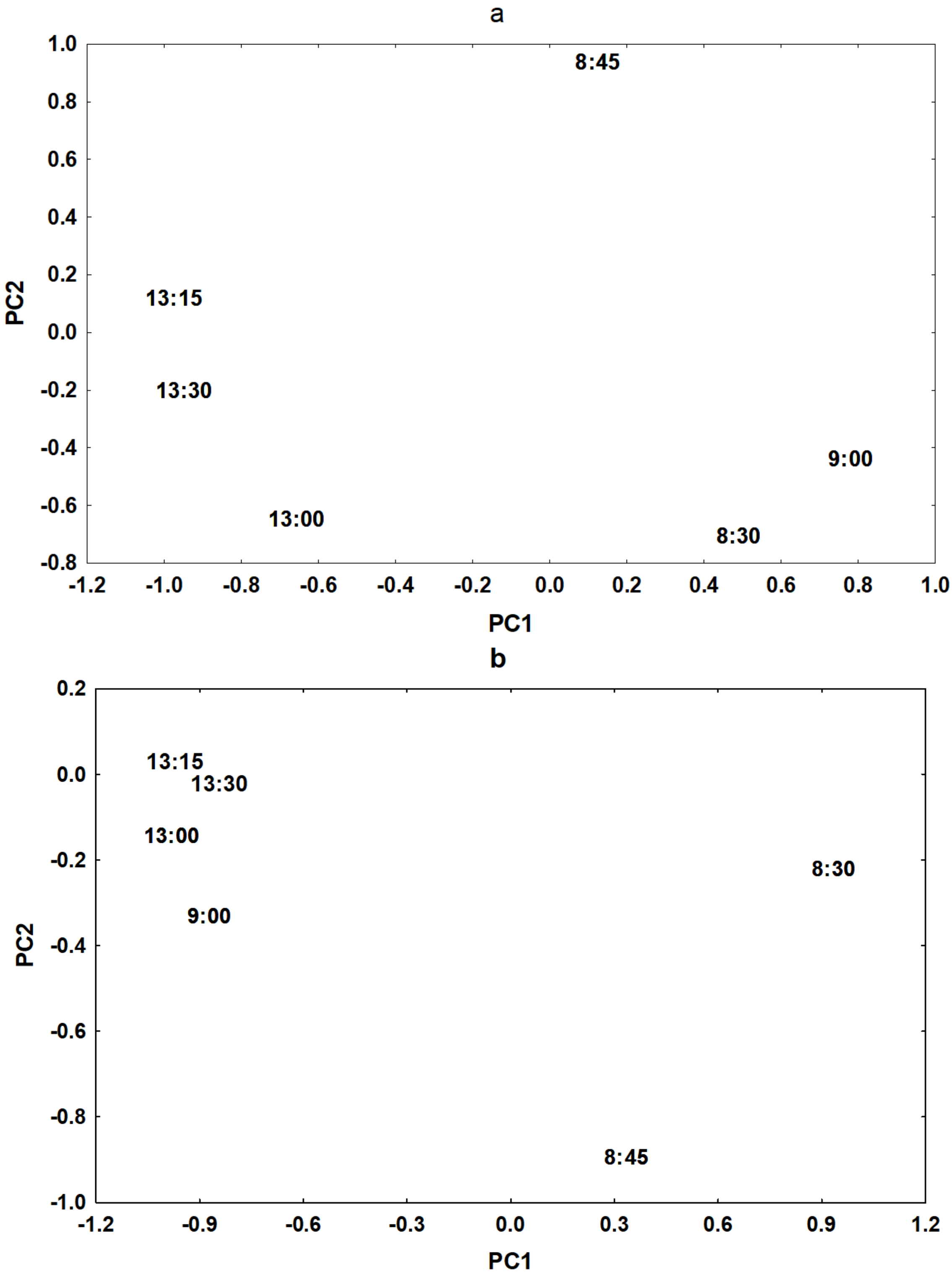

Figure 7. Score plots of the first and second principal components of mechanically damaged dandelion (a) and couch grass (b) leaves collected in the morning and in the afternoon at different times.

4. CONCLUSION

The concentration of different organic compounds in plant leaves varied over a relatively short period of time. These fluctuations could have been caused by natural factors such as circadian rhythms, changes in air temperature and humidity, the geomagnetic field, and NO and O_3 levels in ground-level air. Mechanically induced stress very rapidly led to changes in the concentration of different organic compounds in plant leaves. However, after a short time, 30 minutes, the concentration of the biomolecules in the damaged leaves often returned to the previous state.

Based on Raman spectroscopy data, it can be assumed that under conditions of abiotic stress, plants were forced to quickly adapt to it. Although couch grass and dandelion grew under the same conditions and were collected at the same time, the changes resulting from the damage to plant leaves were often different for these two plant species.

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