User Manual for SpectraCrop Plant Vitality and P-Tester

SpectraCrop

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1. Terms and Conditions

The Plant Vitality and P-Tester is a complicated apparatus using sophisticated algorithms to predict the vitality and P status of plants. Correct interpretation of results requires knowledge to the possibilities and limitations of the technique.

The P-tester is introduced on the market in a tightly controlled program running in the growing seasons of 2017-18, 2018-19, and 2019-20, where only a limited number of devices are offered to selected users. Implementation of a trial and introduction period allows the team behind SpectraCrop to adapt and improve hardware and algorithms behind the P-tester under variable field conditions and in different crop and plant species, for the benefit of all future users.

Purchase of the instrument is therefor offered under the following conditions:

1. Use of the instrument must happen in accordance with the user manual and users must be aware of the limitations of the technique.
2. Data obtained by the P-tester are automatically stored on a server owned by SpectraCrop. To aid in future development and upgrades of hardware and algorithms, users agree that all collected data are available to SpectraCrop. Data will not be handed over to any third-party by SpectraCrop.
3. SpectraCrop cannot guarantee the accuracy of the measurements and cannot be held responsible for any errors or losses occurring as a result of measurements, obtained by the P-tester.
4. SpectraCrop cannot guarantee full functionality of the cloud server and users must accept that down-time periods might occur.
5. Users agree that publication of any result in any format obtained by the P-tester must be approved by SpectraCrop during the trial and introduction period running until the end of the growing season 2019 (31/9-2019). Guidance in use and in interpretation of data during that period is offered as a free service.

2. Introduction

Congratulations on your new Plant Vitality and P-Tester from SpectraCrop. With the P-tester, you can perform measurements on plants directly in the field, and in seconds determine whether the P nutritional status is adequate. The handheld device transmits the readings directly to your smartphone (iPhone). With the use of easily understandable graphics, you get instant answers on essential plant vitality parameters including a P diagnose.

The P-tester records chlorophyll *a* fluorescence which reflects the efficiency of the electron transport in photosynthesis. Based on the course of these fluorescence transients, important information is revealed on general plant vitality and P status (Figure 1).

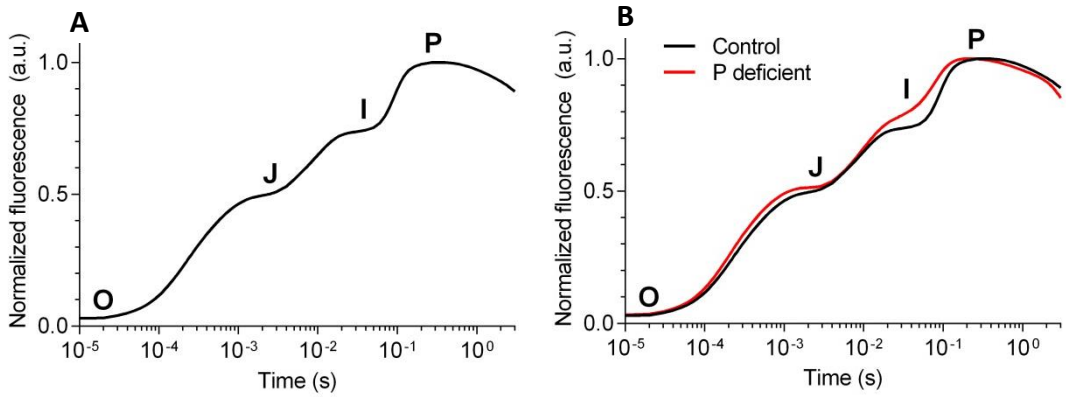

Figure 1: A: A normal chlorophyll *a* fluorescence transient from a healthy plant. B: A fluorescence transient from a healthy control and a P deficient plant. Note that the x-axis is on a logarithmic scale.

The chlorophyll *a* fluorescence transient is also known as an OJIP curve, where base-line fluorescence is known as "O", the two intermediate steps are named "J" and "I" and maximum fluorescence is called "P". A healthy plant will always reveal a transient similar the one in Figure 1A. Every reading is visualized in the SpectraCrop app, and any deviations from the healthy fluorescence transient indicate plant stress.

In addition to commonly used plant vitality measures, the P-tester estimates the bioactive pool of P in the biophysical processes of photosynthesis. Phosphorus has a very important function in the formation of ATP, which is used in the cascades of biochemical processes that fix CO_2 from the air and convert it into carbohydrates. If P is not present in optimal concentration, key processes in

photosynthesis are affected, and so in-turn is the OJIP curve. Identifying this effect in the OJIP curve is what allows the P-tester to specifically, and sensitively, measure the nutritional P-status of a plant. In Figure 1B the fluorescence transient of a healthy control plant and a P deficient plant is illustrated. It is evident that at the so called “I-step” the two curves are easy distinguishable. It is based on the deviations within this area, that the P-tester is calculating the P status of the plant. As the process of photosynthesis is the same for all higher plants, there is no need for correction and calibration in order to adapt the reading to a specific plant species.

For further information regarding the scientific approach used in this device see:

- *Frydenvang et al. (2015): Sensitive detection of phosphorus deficiency in plants using chlorophyll a fluorescence. Plant Physiology, Breakthrough Technologies, vol. 169, pages 353-361.*
- *Carstensen et al. (2018): The impacts of phosphorus deficiency on the photosynthetic electron transport chain. Plant physiology, vol. 177, pages 271-284.*
- *Carstensen et al. (2019): Chlorophyll a fluorescence analysis can detect phosphorus deficiency under field conditions and is an effective tool to prevent grain yield reductions in spring barley (Hordeum vulgare L.). Plant and soil, vol. 434, pages 79-91.*

3. SpectraCrop Plant Vitality and P-Tester

The SpectraCrop Plant Vitality and P-Tester is accompanied with a handy suitcase which includes the following items: Handheld P-tester, 10 leaf clips to dark adapt leaves prior to measurements and 1 power plug (EC standard; Figure 2).

Figure 2: The P-tester is accompanied with a handy suitcase.

3.1 Flow Chart

The flow of data after a measurement with the P-tester is presented in Figure 3. Leaf measurements are transferred via Bluetooth to the SpectraCrop app on your Iphone, where four plant vitality parameters immediately are present. The P-diagnose is calculated on an external server, and will first be available when the data has been sent and received via the app. Note that your phone must send and receive data to obtain the P-diagnose.

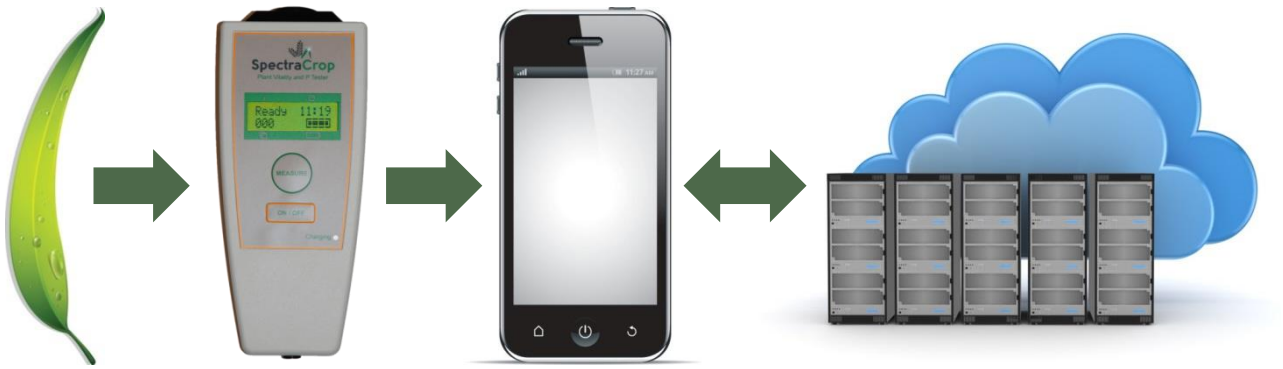

Figure 3: Flow chart of the use of the P-tester. Bluetooth connection ensures transfer of the measurements to the SpectraCrop app, whereas the phone must send and receive data to obtain the P-diagnose from the external server.

4. How to Perform a Measurement

Dark adaption:

The leaf needs to be dark adapted for a minimum of 25 minutes prior to performing the measurement. Mount the leaf clip – **OBS: Remember to close the shutter when the clip is positioned correctly** - on the midsection of a leaf to dark adapt the area that will be probed by the P-tester. This can be done directly on the plant without detaching the leaf (Figure 4).

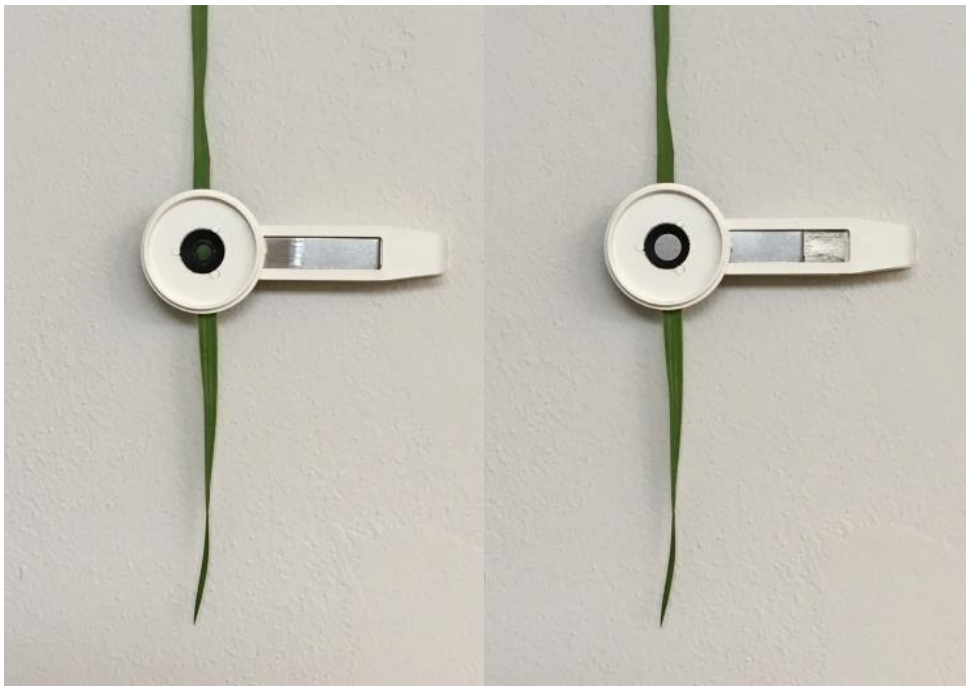

Figure 4: Left: Mounted leaf clip with open shutter. Right: Mounted leaf clip with closed shutter to begin dark adaption period.

Perform measurement:

After a >25 minutes dark adaption the measurement can be performed (not a problem if it takes longer). Place the device onto the clip **with the shutter closed**. After a tight fit between clip and device has been obtained, open the shutter and perform the measurement by pressing “measure” on the P-tester. The measurement will take 3 seconds (Figure 5). You can remove the leaf clip when “measuring” is no longer displayed. The measurement is saved on the P-tester until it is transferred to the SpectraCrop app.

Figure 5: The P-tester attached to a leaf clip and ready for measurement. It is critical that the shutter is not opened before a tight fit between the device and the clip is obtained.

5. The SpectraCrop App

The SpectraCrop app is acquired free of charge from the Apple App Store by searching for 'SpectraCrop' in the App store search pane. **Notice that the app is currently only available for iPhone and iPad**.

When opening the app, you will see four main menu-items along the bottom of the screen: "New", "List", "Map", and "User" (Figure 6).

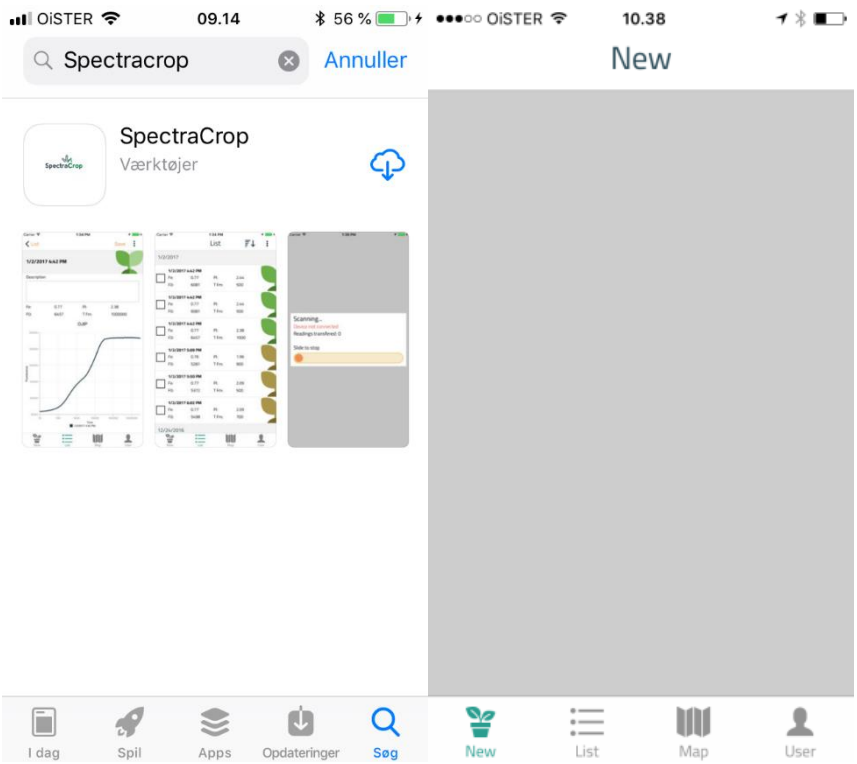

Figure 6: Left: SpectraCrop app from Apple App store. Right: The appearance of the app including the 4 menus at the bottom.

5.1 New

In the "New" menu, you can establish Bluetooth connection with the P-tester. Make sure to turn on Bluetooth from your phone and press "Find device". A few seconds later, the Bluetooth connection is confirmed (Figure 7).

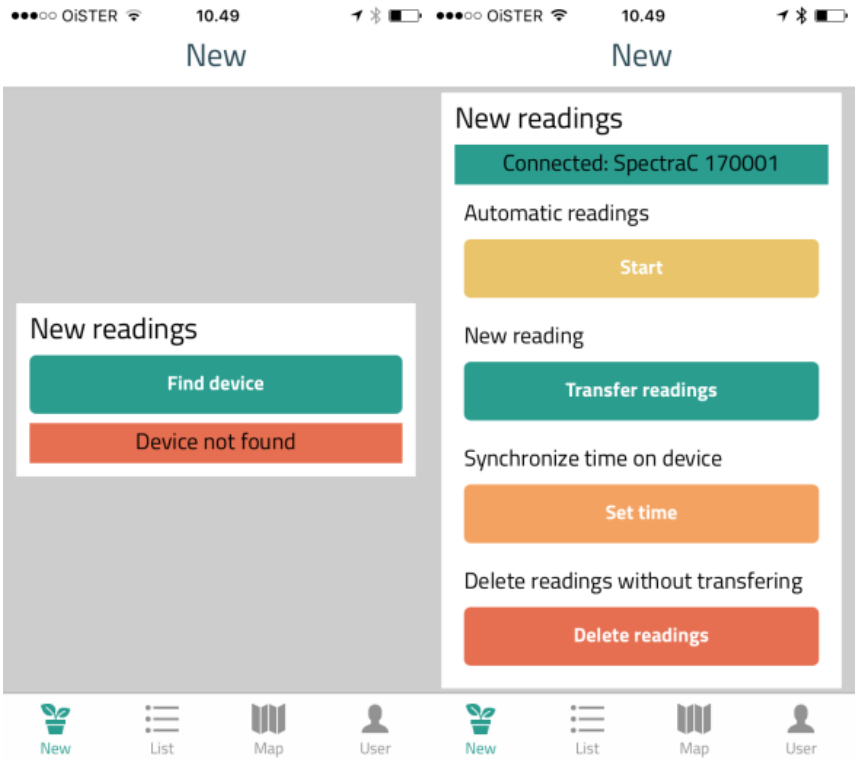

Figure 7: Left: The appearance when Bluetooth connection is not established. Right: After establishment of Bluetooth connection.

Five actions are made available after Bluetooth connection:

Automatic readings: If the "Start" button is pressed under automatic readings, the app will transfer any reading from the P-tester every 10 seconds until stopped (Figure 8). When a reading is transferred it will be notified on the app. **Please note that if you perform a new measurement while the app is transferring the prior reading, it might delay the transferring**. It is therefore recommended to wait until transferring of a reading is finished, before taking a new one. When data is transferred to the phone, the GPS location of the phone is assigned to the measurements. Note that if multiple readings are transferred at the same time, they will obtain the same GPS recording. When a reading is successfully transferred to the app, it will automatically be deleted from the P-tester.

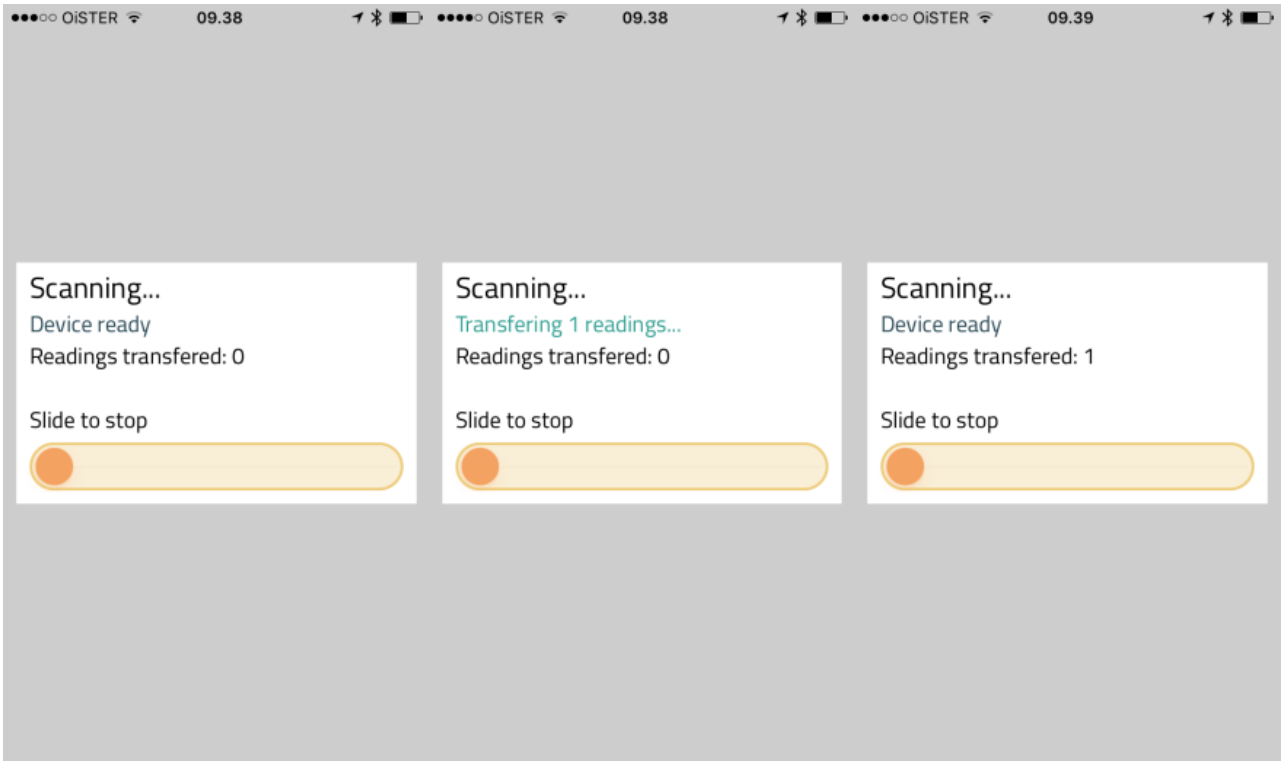

Figure 8: Left: Screenshot when the app is set for automatic reading. Middle: Screenshot when the app is transferring a reading. Right: Screenshot after the reading has been transferred. Note that the automatic reading is still in progress.

New reading: If you don't want to transfer your readings continuously, or you don't have the phone near you when measuring, you can transfer your readings manually by pressing the "Transfer readings" button. **Please note it will take approximately 5.5 seconds for each measurement to be transferred.** If you have 20 measurements it will take close to two minutes, 50 measurements will take 5 minutes, 100 measurements will take 9 minutes and 200 measurements will take 18 minutes for completing the transferring. Note that GPS recording is performed by the Iphone, so all the readings you transfer manually, will be assigned the GPS coordinates of your phone at the time you transfer the readings. After a successful transfer, you can either keep your measurements on the P-tester or delete them.

Synchronize time on device: Press "Set time" to synchronize the time on your phone with the P-tester. Usually only necessary prior first-time use.

Delete readings without transferring: If you want to delete all readings on the P-tester without transferring them to the app press "Delete readings".

Reset current connection with device: If the connection is unstable you can press “Reconnect”, to re-establish Bluetooth connection with the P-tester.

5.2 List

In the “List” menu, all the transferred readings are listed and ready for inspection. Two plant vitality parameters are immediately available: Photosynthetic efficiency (PE) and plant vitality-index (PI), including two additional parameters describing the fluorescence transient: Absolute difference (AD) and Correlation (Cor). Finally, the P diagnose is revealed by the colour of the plant leaf at the far right (Figure 9). Note that the P diagnose is calculated on a server, and will first be available when the data has been sent and received via the app.

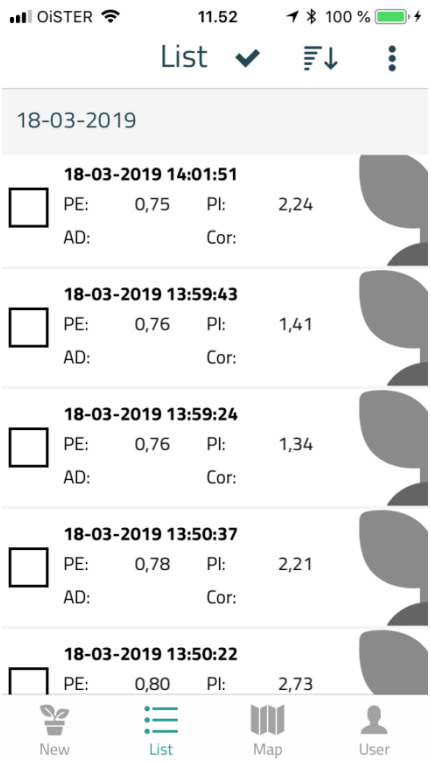

Figure 9: The appearance of a newly transferred reading in the list menu. Note the two immediately available plant vitality parameters (PE and PI). The AD and Cor parameter including the P diagnose (the grey colour of the leaf to the far right) is yet to be calculated.

Photosynthetic efficiency (PE): This parameter is one of two important plant vitality parameters. In a non-stressed plant, regardless of plant species, the value of PE ranges between 0.75-0.85. If

the value is less than 0.75, it reveals that the light is not used optimally in photosynthesis and plant growth is weakened as a result. If PE remains low over time, the plant will become susceptible to diseases, develop leaf symptoms (necrosis) and crop yield and quality of the crop will decrease. PE is an unspecific parameter that provides no information on which stressor might affect the photosynthetic activity, but it provides an overall indication of whether the plant is healthy or not. If the value is less than 0.75 a systematic approach for finding the cause of the plant stress must be implemented and initiatives must be taken to restore vitality through good farming practice. Photosynthetic efficiency is a very useful and sensitive parameter that provides the opportunity to follow plant vitality and optimize the growth conditions, and is one of the strongest parameters, the plant grower have available to optimize productivity. If the PE value is below 0.65, the plant is too affected to allow a P diagnosis and no data will be returned from the server, as it will not reflect the true P concentration in the leaf.

Plant vitality-index (PI): This parameter represents an additional plant vitality parameter, and is a measurement of the energy transfer between the two photosystems (photosystem II and I) in plants. The PI value of a healthy plant will always be larger than 2.5 - If the value is smaller, it indicates that the plant is exposed to stress – The origin of the stressor cannot be predicted. Often the PI value follow PE (see above), but PI has been shown to be more sensitive to certain kinds of stress, such as drought and heat. Therefore, PI is a very useful additional parameter to PE, in order to optimize plant productivity.

Absolute difference (AD): This parameter reveals the difference between the measured OJIP transient and a standard OJIP transient, which is used as an internal reference. The reference OJIP transient is further present in the chart function of the app, which allows a quick visual evaluation of the quality of the taken measurements. The AD value is used as a quality check of the measurements. If the value is above 120, the current measurement deviates too much from a reference OJIP transient. These measurements will be classified as bad measurements, and will not receive a nutritional P status, but assigned with a purple colour. Plants exposed to other stressed than P deficiency, including photosynthetic stress and high temperatures, might increase the AD value.

Correlation (Cor): This parameter describes the correlation between the measured OJIP transient and the reference OJIP transient. The parameter is used as a quality control of the obtained measurements. If the value is below 0.987, the deviation from the reference OJIP transient is too large to obtain a reliable P diagnosis. These measurements will be classified as bad measurements, and will be assigned with a purple colour. Plants exposed to other stressed than P deficiency, including photosynthetic stress and high temperatures, might decrease the Cor value.

P diagnosis: To obtain a diagnosis of the P nutritional status, the readings need to be transferred to an external server via the app (see more under the "user" menu). After receiving a P diagnosis from the server, the result will be displayed with different colours of the plant leaf (Figure 10). At first, the leaf will appear grey, which means that the P diagnose hasn't been calculated yet. After the readings have been processed by the server, the P diagnose will be divided into 3 categories: Green colour means healthy, yellow colour means intermediate and red colour means P deficient.

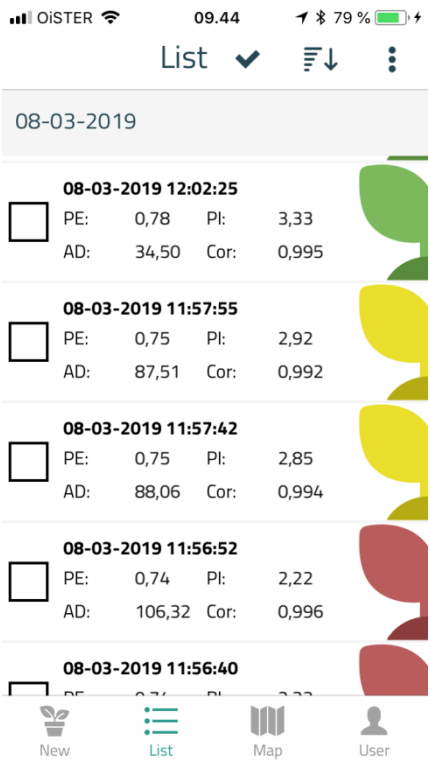

Figure 10: The three different P diagnoses. Green colour means healthy, yellow colour means intermediate and red colour means P deficient.

Bad measurements: The three parameters PE, AD and Cor are used to evaluate if the fluorescence transient is suitable for further analysis on the server to obtain a P diagnosis. If PE is below 0.65, and/or AD is above 120, and/or Cor is below 0.987, the font of the parameters turn red and a P diagnosis will not be performed. Instead, the leaf colour will turn purple to indicate that a bad measurement has been performed (Figure 11).

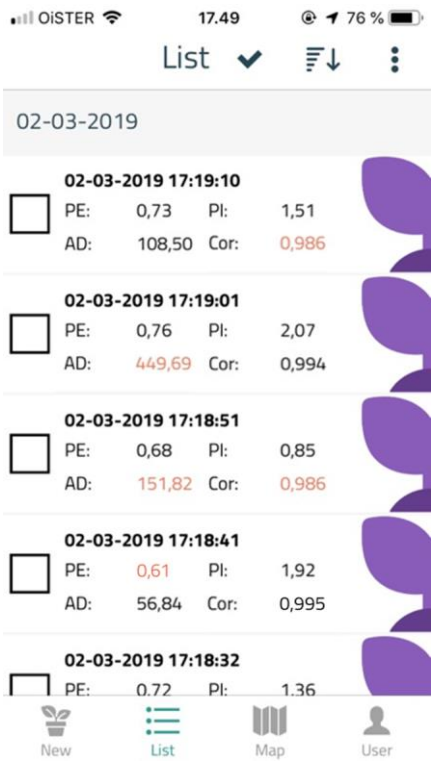

Figure 11: Here Both PE, AD, and Cor are off limit and highlighted in red. This implies a bad measurement, and the leaf colour turns purple, meaning that the P diagnose cannot be performed.

Climatic conditions as high temperatures followed by high solar radiation are known to have an impact on the three parameters. If multiple purple coloured measurements are obtained, it might be necessary to perform the measurements early morning or late afternoon.

The Kautsky curve: The Kautsky curve can be closely inspected by pressing on each individual reading (Figure 12). Please note that by turning your phone sideways, the plot will change accordingly, making it easier to compare individual curves. The used reference OJIP transient will always be present in the plot, making it easier to evaluate the quality of the obtained measurements.

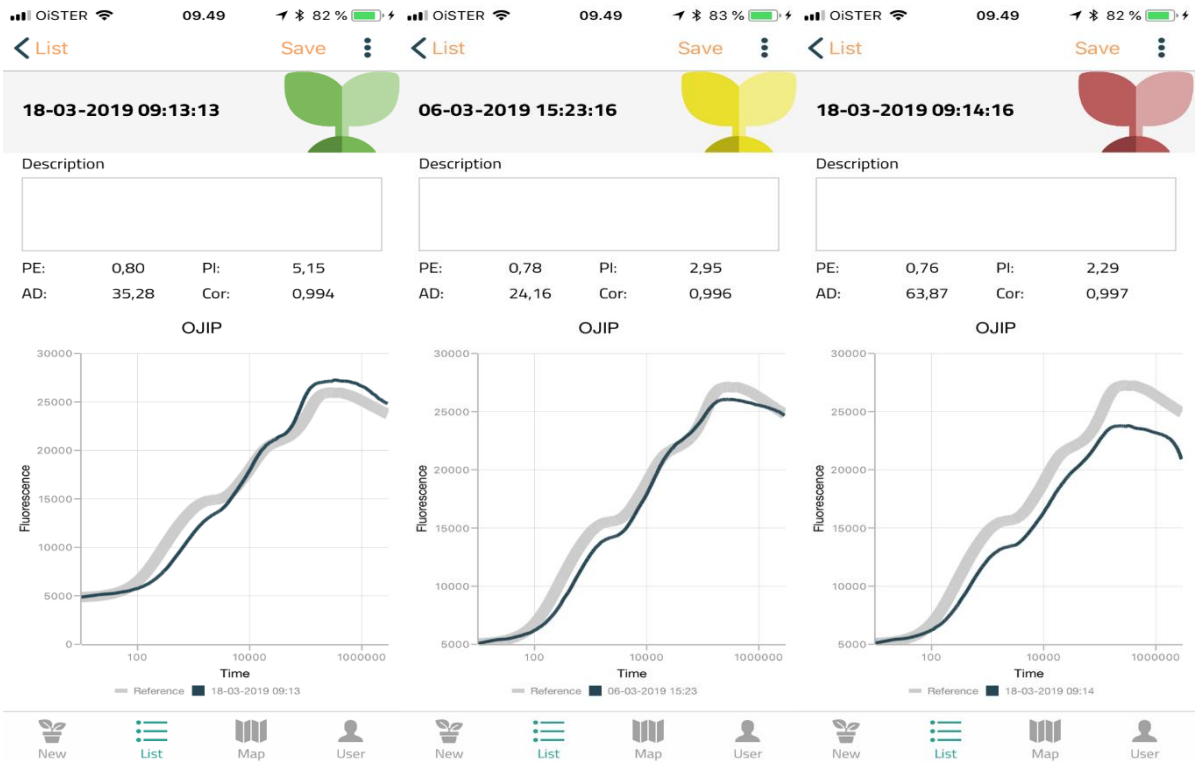

Figure 12: Screenshot of the Kautksy curve off three different readings with corresponding P diagnose. Note the difference at the I-step which corresponds to the three different P diagnoses. A reference OJIP transient is always present as a thicker grey line.

<u>Handling of data:</u> You can mark individual readings by ticking the square box to the left of each reading, and you can mark all the readings of a specific day by pressing the grey date line in the List menu. To mark/unmark all readings you can press "✔" in the upper panel. To sort data, press "⇶" in the upper right. This enables you to sort the readings by either date or P diagnosis (Figure 13). By pressing "⋮" a number of actions are made available. Marked readings can be shown on a map, or the OJIP transients can be compared by the chart function. The values can furthermore be recalculated, the GPS marking can be changed to your current location, and you can export your readings as a .csv file. When reading the .csv file in a data handling program, please note to set the x-axis to a logarithmic scale (log10), if visualization of the curves are needed. Finally, you can delete the marked readings.

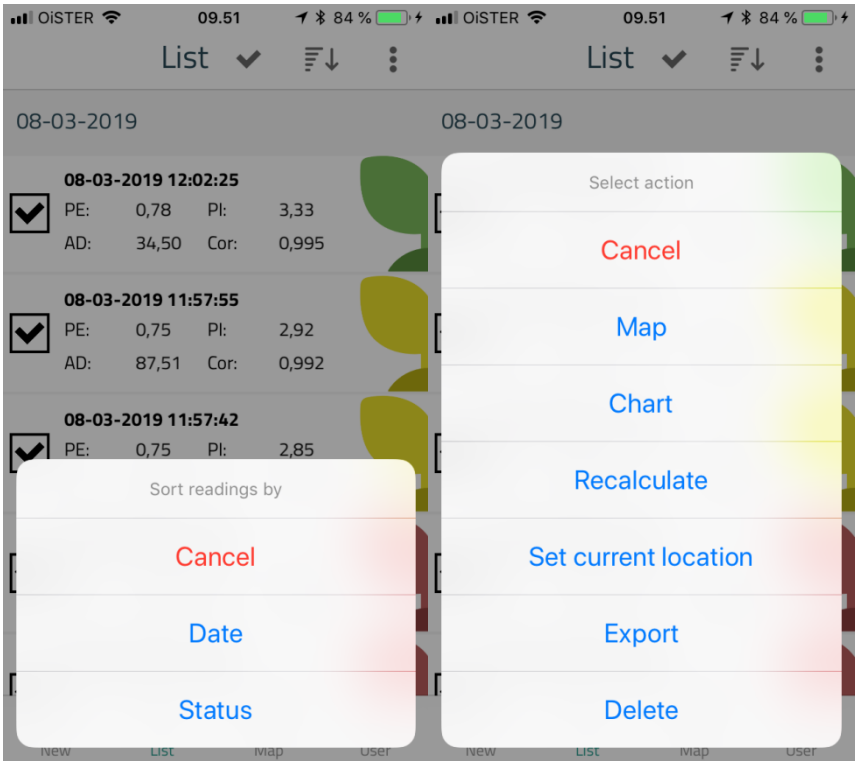

Figure 13: Screenshots to sort data (left) or perform different actions with the marked readings (right).

5.3 Map

In the "Map" menu, the readings appear based on their associated GPS coordinates, including the colour code from the P diagnose (Figure 14). This gives a quick overview of potential local variations in the P availability.

Figure 14: Screenshot of seven readings plotted on a map by their GPS recording. The map-function reveals a potential P problem in the corner of the field.

5.4 User

In the “User” menu, you can get access to the server and synchronize your readings to obtain the P diagnosis. As a first-time-user you need to create a user profile to connect to the server. Press the “Synchronize readings” button and wait a moment to allow synchronization (Figure 15). After a successful transfer, your readings are now updated with a P diagnosis. Note that the phone needs to send and receive data to complete the P diagnosis.

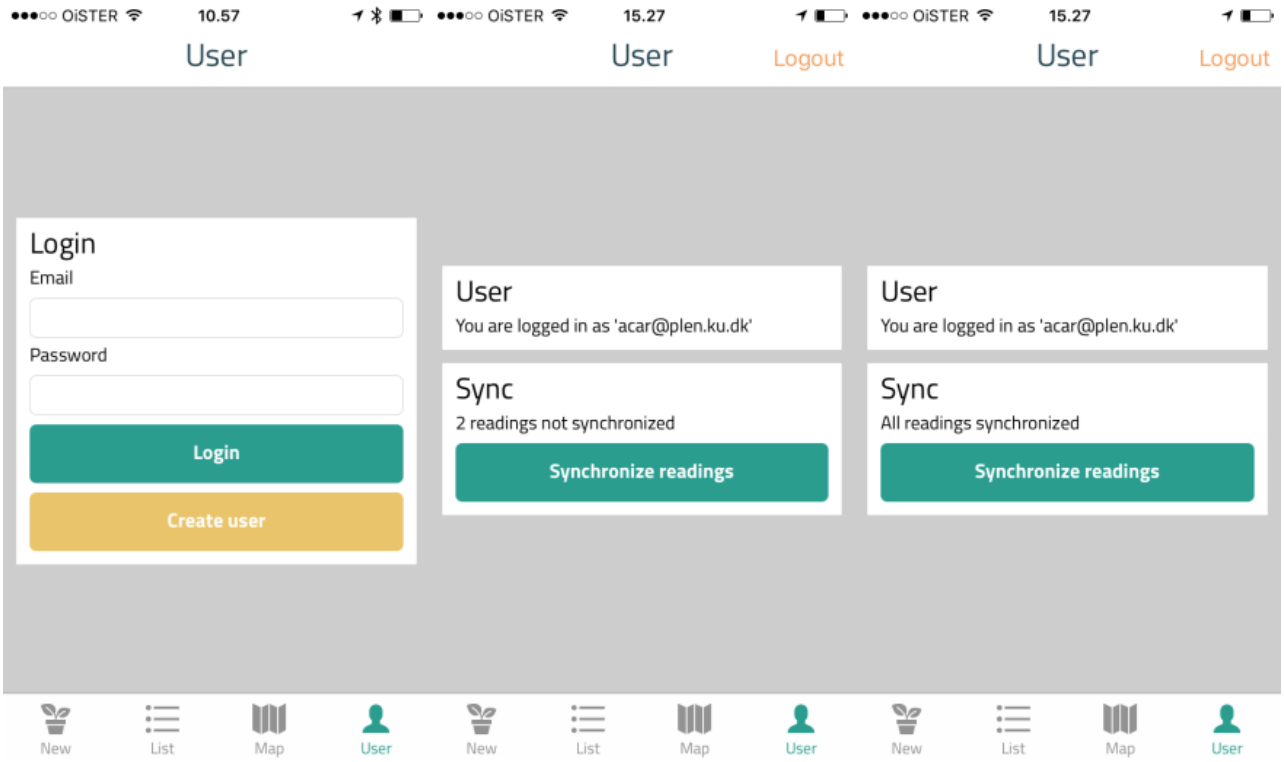

Figure 15: Left: Screenshot of the ”User” menu. As a first time user, you need to create a user before you have access to the server. Middle: Screenshot when you have logged. Right: Screenshot when the synchronization is completed.

6. How to Use the P-tester

- Phosphorus availability is very important during the first month after crop establishment, and it is important to follow the crop closely during this time frame. After approximately one month, the yield potential has been adjusted depending on the growing conditions, and at this point, it is often too late to correct it by additional P fertilization. Especially for grasses (cereals) this is an important time frame, as P availability is essential for the number of tillers and tiller development. In barley, it is important to measure early during the seedling development stage (Zadoks stage 10-15) to get reliable results of true plant P status. The measurements should be performed before the transition from vegetative to generative growth, as the remobilization of P within the plant will result in unreliable measurements of true plant P demand. As a consequence, you might observe plants that clearly show visual P symptoms, also when compared to a control plant, be diagnosed as a healthy plant with optimum P concentrations after this time period. The plants would have revealed P deficiency during the previous weeks, but have now adjusted the growth to the prevalent conditions, and are now capable of completing their life cycle without P deficiency because the biomass has been reduced. It is important to be aware of this basic scenario, which is a general trait in tillering plants to avoid the impacts of P deficiency.
- As P is a mobile plant nutrient, the youngest fully developed leaf will be the most accurate leaf to use for the measurements. However, P deficiency might be recorded earlier if measurements are performed on the second or even the third youngest fully developed leaf. Please note, that when using other leaves than the youngest fully developed, other factors as drought, temperatures, diseases or even senescence might influence the general leaf status, which will result in a bad measurement. The SpectraCrop P-model is optimized for measurements on the youngest fully developed leaf, and we therefore recommend using that leaf when measuring. However, it should be noted that only a limited amount of crop species have been tested so far and it cannot be ruled out that other leaf positions might be relevant or even better indicators of the plant P status.
- As a dark adaption period of minimum 25 minutes is necessary prior to every measurement, the most effective measuring strategy is usually to start dark adapting all your samples first. After 25 minutes you can start measure the plants one by one. The dark adaption period should be *minimum* 25 minutes, and it will have no effect on the measurements, if the leaves are dark adapted additional 10-20 minutes.
- The P dynamics on a single leaf level is still not clarified, however, placing the leaf clip on the leaf midsection is the most common used practice.