Spatial Distribution of Mucilage in the Rhizosphere Measured With Infrared Spectroscopy

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Mucilage is receiving increasing attention because of its putative effects on plant growth, but so far no method is available to measure its spatial distribution in the rhizosphere. We tested whether the C-H signal related to mucilage fatty acids is detectable by infrared spectroscopy and if this method can be used to determine the spatial distribution of mucilage in the rhizosphere. Maize plants were grown in rhizoboxes filled with soil free of organic matter. Infrared measurements were carried out along transects perpendicular as well as axially to the root channels. The perpendicular gradients of the C-H proportions showed a decrease of C-H with increasing distance: 0.8 mm apart from the root center the C-H signals achieved a level near zero. The measured concentrations of mucilage were comparable with results obtained in previous studies, which encourages the use of infrared spectroscopy to quantitatively image mucilage in the rhizosphere.

Keywords: rhizosphere extension, root mucilage, DRIFT spectroscopy, maize (Zea mays L.), soil hydrophobicity

INTRODUCTION

The rhizosphere is the soil volume around the roots and plays a crucial role for nutrient and water acquisition by plants (Gregory, 2006; Neumann et al., 2009). Organic compounds (rhizodeposits) are released through the root into the rhizosphere in different chemical forms and they may have positive effects on plant growth (Jones et al., 2009). Mucilage is one of those compounds and makes up 2–12% of total rhizodeposition (Dennis et al., 2010 and references therein). Mucilage is a gel-like substance and is released from the root-cap cells (Figure 1). Its main components are polysaccharides (∼94%), proteins (1–5%) and phospholipids (Oades, 1978; Bacic et al., 1986; Read et al., 2003; Jones et al., 2009; Carminati and Vetterlein, 2013).

Mucilage provides several benefits for plant growth, such as the lubrication during root penetration (Iijima et al., 2004) or the stabilization of aggregates (Morel et al., 1991; Traoré et al., 2000). Recently it has been shown that mucilage increases rhizosphere water content (Carminati et al., 2010) due to the high water-holding capacity of the contained polysaccharides (McCully and Boyer, 1997) and it may therefore facilitate root water uptake (Ahmed et al., 2014). The extent of such benefits depends on the spatial distribution of mucilage around roots. To date, there is no experimental method to non-invasively and quantitatively image mucilage in soils and our knowledge of mucilage spatial distribution remains largely speculative.
Among the many effects of mucilage on soil properties, one spectacular effect is the rhizosphere water repellency (Hallett, 2003; Whalley et al., 2004; Moradi et al., 2012). While the polysaccharides contained in mucilage have a very high water holding capacity (McCully and Boyer, 1997), the lipids which are also contained (Read et al., 2003), can have a reverse effect when mucilage dries. It is therefore likely that mucilage is responsible for the observed rhizosphere hydrophobicity (Carminati, 2013; Ahmed et al., 2015; Benard et al., 2017). Fatty acids are composed of a hydrophilic and a hydrophobic tail of nonpolar fatty acid chains (C-H groups). Upon soil drying these hydrophobic tails probably turn outwards. Hereby they reduce soil wettability and cause a hydrophobic region around the root as indicated in Figure 2.

Diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy can be used to map functional groups such as C-H groups at intact soil surfaces at the mm-scale (Leue et al., 2010, 2015). In combination with microscopic techniques, DRIFT spectroscopy can be used to determine organic functional groups also at the µm-scale. The aim of this study was to use the C-H groups of the fatty acids contained in mucilage as a proxy for mucilage distribution in the rhizosphere. Although the main compound of mucilage is polysaccharides, we used fatty acids as a proxy for mucilage because the C-H groups are specific for mucilage, while no functional group specifically for polysaccharides exists. Although C=O or O-H groups can be measured by IR spectroscopy, those groups are not specifically for polysaccharides because they are also present in sugar monomers or amino acids, which are present in the rhizosphere as they are released as root exudates.

We used DRIFT spectroscopy and Fourier-transform infrared spectroscopy (FTIR) microscopy to map the distribution of fatty acids from mucilage in soil. Maize plants were grown in rhizoboxes and the signal of the C-H groups was recorded with a high spatial resolution along the roots as well as perpendicular to the roots.

**MATERIALS AND METHODS**

**Plant Growth and Mucilage Extraction**

Quartz material consisting of 70% quartz sand [collected from Duingen (Germany), particle size: 0.1–0.2 mm] and 30% quartz powder (Carl Roth, particle size: < 0.125 mm) was used for the calibration and as a growth medium free of soil organic matter. For the calibration, maize root mucilage was extracted as described in Zickenrott et al. (2016) in a slightly modified way. A mesh of stainless steel (2 mm diameter mesh size) was hanged into a plastic box 15 cm above surface of the box. The box had a size of 30 × 40 × 30 cm and was filled with water to a height of approximately 6 cm (Figure 3A). An aquarium diffuser was placed into the water in the box and connected to a plastic tube which was plugged to pressurized air. The air
was circulated though the diffuser which creates tiny air bubbles which increased the humidity in the box. Maize seeds were sterilized in 10% H$_2$O$_2$ solution for 10 min and placed on the mesh and the box was closed with a plastic lid to maintain high air humidity. After 3–4 days root tips were growing trough the mesh and showed small drops of hydrated mucilage at the tips due to the high humidity in the box (Figure 3A). The mucilage was collected from the tips once a day till the roots reached the water surface after approximately 3 days. The collection of mucilage was done with a syringe by applying negative pressure. Per box and day we collected approximately 6 mg of dry mucilage.

For the calibration, dried quartz material was mixed with different amounts of previously collected mucilage at concentrations of 0.0, 0.025, 0.05, 0.1, 0.25, 0.5, and 1 mg/g (gram of dry mucilage per gram of dry soil). The mixtures were applied on object slides in four replicates and were air-dried. For the mapping of the spatial distribution of mucilage across root channels, maize (Zea mays L.) plants (KWS 2376) were grown in rhizoboxes with an inner size of 10 × 20 × 1.5 cm filled with the same quartz substrate as used for the calibration. Before germination, maize seeds were immersed in a 10% H$_2$O$_2$ solution for 10 min to avoid seed-borne diseases. Prior to plant growth the following nutrients were added per kg of soil: NH$_4$NO$_3$-N: 0.3 g, Ca(H$_2$PO$_4$)$_2$-P: 0.06 g, K$_2$SO$_4$-K: 0.03 g, CaSO$_4$-Ca: 0.05 g, MgSO$_4$-Mg: 0.05 g, H$_3$BO$_4$-B: 2 mg, CuSO$_4$-Cu: 0.03 mg, MnSO$_4$-Mn: 1.5 mg, (NH$_4$)$_2$MoO$_4$-Mo: 0.03 mg, ZnCl$_2$-Zn: 1.2 mg, FeNaEDTA-Fe: 3.6 mg. During plant growth, the water content was adjusted to 20%. Soil water content was checked gravimetrically each day and lost water was added from the top with a syringe. The rhizoboxes were kept at an angle of approximately 55° to make sure that the roots were growing close to the lower side of the rhizoboxes. The temperature in the climate chamber was 25°C during day and 22°C during night, the photoperiod was 14 h and the light intensity was 300 µmol m$^{-2}$ s$^{-1}$. After 4 weeks of growth, plant shoots were cut and the soil samples were dried at 35°C. A low temperature of 35°C was chosen for drying to allow for a drying process close to natural conditions and to avoid physical and chemical changes of mucilage. After drying, the roots were removed from the soil manually so that the channel where root had been growing in remained as a depression in the otherwise even soil surface (Figure 4).

**Diffuse Reflectance Infrared Spectroscopy**

Diffuse reflectance infrared spectroscopy (DRIFT) measurements in the mid-infrared range (wavelength: 2.5–25 µm, wave numbers (WN): 4,000–400 cm$^{-1}$) was applied to the calibration samples at the object slides using a BioRad FTS 135 spectrometer (Cambridge, Massachusetts, USA). Each DRIFT spectrum was recorded by 16 co-added scans with a
spectral resolution of 4 cm$^{-1}$ against a gold background (Leue et al., 2015). At the intact root channels and surrounding substrate, DRIFT spectra were conducted using an Agilent Cary series 600 FTIR microscope (Agilent Cary series 600, Agilent Technologies, Inc., Santa Clara, CA, USA). The measurements were done at three positions, (a) directly at the root tip, 1 cm behind the root tip and 3 cm behind the root tip. Measurement at each position started in the center of the root channel. From there, IR spectra were recorded each 100 $\mu$m perpendicular to the root channel till a distance of 13 mm from the root channel center was reached (Figures 4, 5). The measurements within the root channel (soil that had been in direct contact with the root) are referred to as “root channel” while measurements beyond the root channel border are referred to as “rhizosphere.” The spectra were recorded as 64 co-added scans at a spectral resolution of 4 cm$^{-1}$ in steps of 100 $\mu$m from sample areas of 0.1 x 0.1 mm. For the rhizosphere samples, one root was chosen per plant and spectra were measured at three positions along each root (0, 1, and 3 cm behind the tip). After preprocessing of the spectra (Ellerbrock et al., 2009; Leue et al., 2010), the local peak heights of the C-H bands were normalized for the absolute peak height of the SiO$_2$ band at WN 1,350 cm$^{-1}$. The relationship between mucilage content and C-H signal intensities obtained from the calibration samples was used to quantify the mucilage content in the rhizosphere of the maize roots. After calibration, the background signal (i.e., the average signal in the bulk soil where no mucilage was expected) was subtracted from each measured value.

**STATISTICS**

To test for differences between soil regions and root positions we applied a one-way ANOVA ($p < 0.05$) followed by a post-hoc test. Statistical analysis were done in R 3.3.1.

**RESULTS**

The calibration measurements on the object slides revealed a linear relationship between mucilage concentration and the C-H/SiO$_2$ ratio. The fit of the linear equation was good and resulted in:

$$y = 0.0299x - 0.0003$$

where $R^2 = 0.99$.

The displayed values are the means of 5 replicates (i.e., plants). Variation is given as standard error. Differences between the positions and the soil regions were not significant ($p < 0.05$).
in an $R^2$ of 0.99 (Figure 6). The calibration equation was applied to the IR measurements at the root channels to obtain mucilage concentration (mg/g).

The IR measurements at the 3 root positions showed that mucilage concentration slightly increased with increasing distance from the root tip (i.e., increasing root age), however, differences between the positions were not statistically significant (Figure 7). Mucilage concentration in the rhizosphere was lower compared to the root channel for all 3 positions but these differences were likewise not statistically different probably due to very high variation between the samples.

The highest mucilage concentrations of 0.02 mg/g were found in the center of the root channel (Figure 8). Toward the border of the root channel this concentration decreased to around 0.01 mg/g. The radial gradients of mucilage from the center of the root channel toward rhizosphere dropped rapidly and decreased to approximately 0 mg/g at a distance of around 0.8 mm from the center of the root channel (Figure 8). Averaging the mucilage concentration in the root channel for all 3 measured root positions resulted in a mucilage concentration of 0.017 mg/g. The rhizosphere reached on average a much lower concentration of 0.003 mg/g mucilage (Figure 9). Differences between both measurements were not different statistically, presumably because of the high variation in the root channel measurements.

**DISCUSSION**

This pilot experiment showed that infrared spectroscopy can in principle be applied to detect gradients of mucilage around roots grown in soil. Although not significant, the measurements of mucilage at different root positions showed an increase in C-H groups (i.e., hydrophobicity) with increasing root age (Figure 7). This is in agreement with Carminati (2013) who found that rhizosphere hydrophobicity was enhanced with increasing root age. This observation was explained by an increased stiffening of mucilage either due to (a) repeated drying and rewetting, (b) interaction between mucilage and solutes such as Ca$^{2+}$ present in soil solution or (c) microbial decomposition of mucilage (Carminati, 2013; Carminati and Vetterlein, 2013). The first two processes cannot explain an increase in C-H groups that we observed. However, it is possible that mucilage was exposed to microbial decomposition. This could cause a change in the ratio of fatty acids to other compounds such as polysaccharides.
or may increase the amount of fatty acids. A change in mucilage composition due to aging would be problematic for the interpretation of our results. As the measurements were calibrated with maize root mucilage, a prerequisite for this approach is that the chemical composition of mucilage used in the calibration is similar to those found in the soil around roots. The fact that we found a trend of increasing C-H signal with increasing root age but no statistical differences, can have two reasons. First, natural variability between plants may be high. This could be solved by a higher number of replicates. Second, the measured root channel segments at distances of 0, 1, and 3 cm behind the tip may have been too young to show differences in mucilage concentration and distribution. Probably longer time intervals (i.e., older root segments) would be necessary to detect changes in mucilage quantity or quality due to decomposition.

The radial gradients of mucilage were relatively steep: at 0.6 mm distance from the border of the root channel, mucilage content decreased below the limit of detection. The extent of the root channel was exposed to microbial decomposition which might have changed its chemical composition. This may be true for mucilage close to the root tip but may be problematic at older root sections where mucilage was not exposed to microbial decomposition which might have changed its chemical composition.

Bearing those aspects in mind, IR spectroscopy can be applied to measure the spatial distribution of mucilage in soil. Future studies could investigate the effect of factors such as: root hairs, root age, root type, plant species, soil texture, and soil water content on the spatial distribution of mucilage in the rhizosphere.
AUTHOR CONTRIBUTIONS

MH, ML: planning and conducting the experiment, data analysis, writing manuscript; MA, PB: planning and conducting experiment; HG: planning experiment, providing facilities for IR measurements; AC: planning experiment, providing funding.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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