FTIR-ATR spectroscopy – a new approach in root discrimination of crop and weed species

FTIR-ATR Spektroskopie – ein neuer Ansatz zur Wurzelunterscheidung von Nutzpflanzen- und Unkrautarten

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Summary

Crop and weed species often compete for the same resources. To analyse below-ground competitive processes, crop and weed roots have to be distinguished from one another. Up to now, a reliable and easy method for plant root discrimination does not exist. In a recent study, Fourier transform infrared (FTIR) spectroscopy with an attenuated total reflection (ATR) device was successfully applied in root discrimination of distantly related plant species (pea/oat). In this experiment, we wanted to test the potential of FTIR-ATR spectroscopy to discriminate roots of closely related crop/weed-combinations. In a greenhouse experiment, two crop and associated weed species were cultivated: Maize/barnyard grass (*Zea mays*/*Echinochloa crus-galli*) and sugar beet/common lambsquarters (*Beta vulgaris*/*Chenopodium album*). To allow inter- and intra-specific competition, plants were grown sole and in crop/weed-combinations. Six weeks after sowing, root biomass was harvested and rinsed with water to remove soil particles. The absorbance patterns of fresh and dry rootlets were recorded by FTIR-ATR spectroscopy. Spectra of fresh rootlets within one plant family showed similar peak distribution, while dry rootles differ in peak location and height. Cluster analyses grouped the absorbance patterns of the dry crop and weed roots according to their similarity and revealed a complete root discrimination of crop and weed species.

Keywords: *Beta vulgaris*, *Chenopodium album*, *Echinochloa crus-galli*, spectral distribution, *Zea mays*

Zusammenfassung

Nutzpflanzen und Unkräuter konkurrieren häufig um die gleichen Ressourcen. Eine Voraussetzung zur Untersuchung von unterirdischer Konkurrenz ist, dass Wurzeln von Nutzpflanzen- und Unkrautarten unterschieden werden können. Eine schnelle und einfache Methode hierzu existiert bisher nicht. In einer früheren Studie wurde Fourier Transform Infrarot (FTIR) Spektroskopie mit abgeschwächter Totalreflektion (ATR) erfolgreich zur Wurzelunterscheidung bei entfernt verwandten Pflanzenarten (Erbse/Hafer) angewendet. In diesem Experiment wollten wir das Potenzial der FTIR-ATR Spektroskopie zur Wurzelunterscheidung bei nahverwandten Nutzpflanze/Unkraut-Kombinationen testen. In einem Gefäßversuch wurden Mais/Gemeine Hühnerhirse (*Zea mays*/*Echinochloa crus-galli*) sowie Zuckerrübe/Weißer Gänsefuß (*Beta vulgaris*/ *Chenopodium album*) angezogen. Um inter- und intraspezifische Konkurrenz zu ermöglichen, sind die Pflanzen einzeln und in Nutzpflanze/Unkraut-Kombinationen gewachsen. Sechs Wochen nach dem Säen wurden Wurzelsegmente geerntet und mit Wasser von Erdpartikeln gereinigt. Die Absorptionsmuster der frischen und getrockneten Wurzelstücke wurden mittels FTIR-ATR Spektroskopie erfasst. Die Spektren der frischen Wurzeln einer Pflanzenfamilie zeigten ähnliche Peakverteilungen und Peakhöhen, während getrocknete Wurzelstücke unterschiedliche Absorptionsmuster aufwiesen. Eine Clusteranalyse gruppierte die Spektren hinsichtlich ihrer Ähnlichkeit. Diese Analyse zeigte eine komplette Auftrennung der getrockneten Wurzelstücke entsprechend ihrer Artzugehörigkeit.

Stichwörter: *Beta vulgaris*, *Chenopodium album*, *Echinochloa crus-galli*, spektrale Verteilung, *Zea mays*

1. Introduction

Plant species often compete for the same resources and much of the competition takes place belowground (CASPER and JACKSON, 1997). Below-ground competition for nutrients and water can show even stronger effects than above-ground competition for light (WILSON, 1988). To analyse below-ground competition processes of crop and weed species, roots have to be distinguished according to species level. Up to now, a reliable and easy method for plant root discrimination does not exist for non-woody plant species.

Fourier transform infrared (FTIR) spectroscopy irradiates the sample by mid-infrared radiation. The

absorption of the sample is recorded and results in a spectrum which represents the chemical composition like a spectral fingerprint. This method has been used for species discrimination of bacteria and fungi (NAUMANN et al., 1991, 2005; MARIEY et al., 2001; NAUMANN, 2009). FTIR spectroscopy, combined with attenuated total reflection (ATR), was recently applied successfully in root discrimination of pea and oat (NAUMANN et al., 2010).

In this study we want to test FTIR-ATR spectroscopy to discriminate roots of closely related crop/weed-combinations.

2. Materials and methods

2.1 Plant material

In a greenhouse experiment, maize (*Zea mays* L.), cultivar "RICARDINIO" and barnyard grass (*Echinochloa crus-galli* L.), provenance Göttingen, were grown as representatives of the plant family Poaceae. As Chenopodiaceae species, sugar beet (*Beta vulgaris* subsp. *vulgaris* var. *altissima* DÖLL.), cultivar „ISABELLA KWS" and common lambsquarters (*Chenopodium album L.*), provenance Göttingen, were cultivated. Plants were sown in a sand-compost (50/50 %) mixture in single species pots (11 x 11cm) with two plants per pot and in mixtures with one crop and one weed species per pot (Tab. 1). Three replicates of crop/weed-combinations in single and mixture pots were randomly distributed within the greenhouse. Maize/barnyard grass and sugar beet/common lambsquarters were cultivated under natural light conditions from March - May 2011 for 33 days and 55 days, respectively. Maize was harvested at BBCH (MEIER, 1997) 15-16 and barnyard grass at BBCH 23. Sugar beet was classified as BBCH 27-28, while common lambsquarters reached BBCH 59-63. Air temperature during growing period ranged from 10.5 °C to 37.5 °C and soil temperature averaged at 20 °C.

Roots of harvested plants were rinsed with a soft waterjet to remove soil particles. In total, six rootlets per plant were selected and placed in 2 ml reaction tubes. Two rootlets of 1 cm length were collected at the basis of the root system, in the middle section of a root and at root tips. Additionally, taproot segments were collected from sugar beet and common lambsquarters. One of the two root segments were dried immediately at 50 °C for two days before spectra were recorded by FTIR-ATR spectroscopy. The second root segment was freshly subjected to FTIR-ATR spectroscopy and dried afterwards.

Tab. 1 Species combination, BBCH stage at harvest, date of harvest and number of replicates.

Tab. 1 *Artenkombinationen pro Topf, BBCH-Stadium bei der Ernte, Erntetermin und Anzahl der Wiederholungen.*

Species combination	BBCH at harvest	Date of harvest	Replicates
Maize/maize	15-16	2 May 2011	3
Barnyard grass/barnyard grass	23	2 May 2011	3
Maize/barnyard grass	15-16/23	2 May 2011	3
Sugar beet/sugar beet	27-28	24 May 2011	3
C. lambsquarters/c. lambsquarters	59-63	24 May 2011	3
Sugar beet/c. lambsquarters	27-28/59-63	24 May 2011	3

2.2 FTIR-ATR spectroscopy

Spectral analysis was accomplished by a FTIR spectrometer (Alpha, Bruker Optics, Ettlingen, Germany) with an ATR devise (diamond crystal). The root segments were placed on top of the ATR crystal, the infrared beam is totally reflected at the interface between the sample and the crystal. At the interface, the radiation interacts with the sample and is attenuated (approximate penetration depth into the sample: 1-2 µm). Spectra were recorded with a resolution of 4 cm^{-1} and 64 scans in the spectral range of 4000-400 cm^{-1}. The FTIR spectrum is calculated from the attenuated beam and displayed as absorbance against wavenumber (cm^{-1}).

Cluster analyses were conducted with the software OPUS (Version 6.5, Bruker Optics, Ettlingen, Germany). Spectra were pre-processed by calculating the first derivative, vector-normalization and offset-correction. Cluster dendrogram was constructed by means of Euclidian distance and Ward's

algorithm. The differences of spectral patterns were expressed as heterogeneity.

3. Results

3.1 Spectral patterns of roots

The spectra of dry roots showed similar peak distribution between wavenumber 400 cm^{-1} and 1800 cm^{-1}, reaching the highest absorption rates at 3292 cm^{-1} and 3328 cm^{-1}. Furthermore, all species demonstrated a peak from 2921-2818 cm^{-1} (Fig. 1). Peak distribution and height differed among species from 1800-400 cm^{-1}. All species had a maximum peak between 1030 cm^{-1} and 1028 cm^{-1}.

Dry rootlets of maize and barnyard grass demonstrated the highest absorption rate of tested species at 3328 cm^{-1} and 1030 cm^{-1}. In contrast to sugar beet and common lambsquarters, maize and barnyard grass spectra differed in peak location. Barnyard grass root spectra showed a characteristic peak at 1637 cm^{-1} while maize root spectra revealed a distinct peak at 1571 cm^{-1} (Tab. 2).

Tab. 2 Absorption peaks of dry rootlets of maize, barnyard grass, sugar beet, and common lambsquarters. Characteristic peaks are highlighted in bold numbers.

Tab. 2 *Absorptionspeaks von getrockneten Mais-, Hühnerhirse-, Zuckerrübe- und Weißer Gänsefußwurzeln. Fett geschriebene Wellenzahlen markieren charakteristische Absorptionspeaks.*

Species	Absorption peaks (wavenumber cm^{-1})
Maize	3328, 2918, **1571**, 1369, 1245, 1028
Barnyard grass	3328, 2919, **1637**, 1368, 1246, 1030
Sugar beet	3292, 2921, 1619, **1415**, **1319**, 1030
Common lambsquarters	3308, 2919, **1733**, 1623, 1371, 1319, **1240**, 1028

Spectra of dry sugar beet and common lambsquarters roots showed similar peak location except between wavenumber 1800 cm^{-1} and 1200 cm^{-1}. In this range, common lambsquarters roots offered absorption peaks at 1733 cm^{-1} and 1240 cm^{-1}, whereas sugar beet roots presented a peak at 1415 cm^{1}.

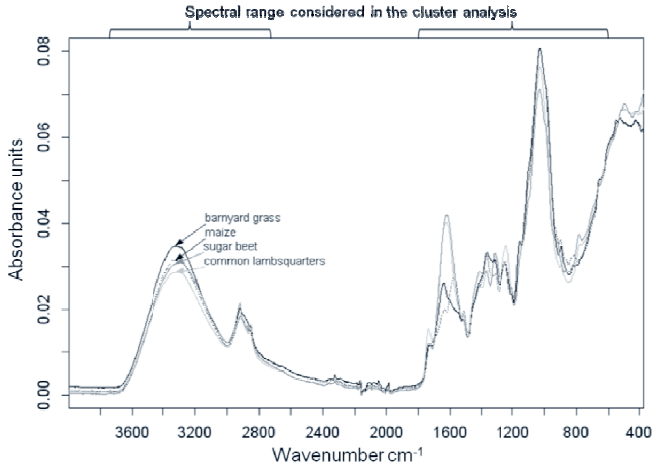

Fig. 1 Mean FTIR-ATR spectra of dry roots of maize, barnyard grass, sugar beet, and common lambsquarters. Means are calculated of three (root basis, middle, and tip section) and four (plus taproot) root segments of nine individuals per species. Data are vector-normalised and offset-corrected. Brackets indicate the spectral range considered in the cluster analysis.

Abb. 1 *Absorption der FTIR-ATR-Spektren von getrockneten Mais-, Hühnerhirse-, Zuckerrübe- und Weißer Gänsefußwurzeln. Die Mittelwerte der Spektren wurden gebildet aus drei (Wurzelbasis, -mitte und -spitze), bzw. vier (Pfahlwurzel) Wurzelsegmenten der jeweiligen Arten mit neun Individuen pro Art. Die Daten sind vektornormiert und offset-korrigiert. Die Klammern kennzeichnen den spektralen Bereich, der in der Clusteranalyse berücksichtigt wurde.*

3.2 Root discrimination by cluster analysis

Similarities of spectra were analysed by cluster analysis. Cluster analysis of spectra from maize and barnyard grass roots revealed a separation according to species (Fig. 2A). The intra-specific heterogeneity of barnyard grass was 1.18 and 2.30 in maize. The inter-specific heterogeneity of maize and barnyard grass was 3.98 and therefore much higher than the respective intra-specific one. A distinct separation of roots according to species was displayed by cluster analysis of sugar beet and barnyard grass root spectra. Sugar beet showed intra-specific heterogeneity of 1.97 and common lambsquarters grouped at 2.24, which is much lower than their inter-specific heterogeneity of 6.34 (Fig. 2B).

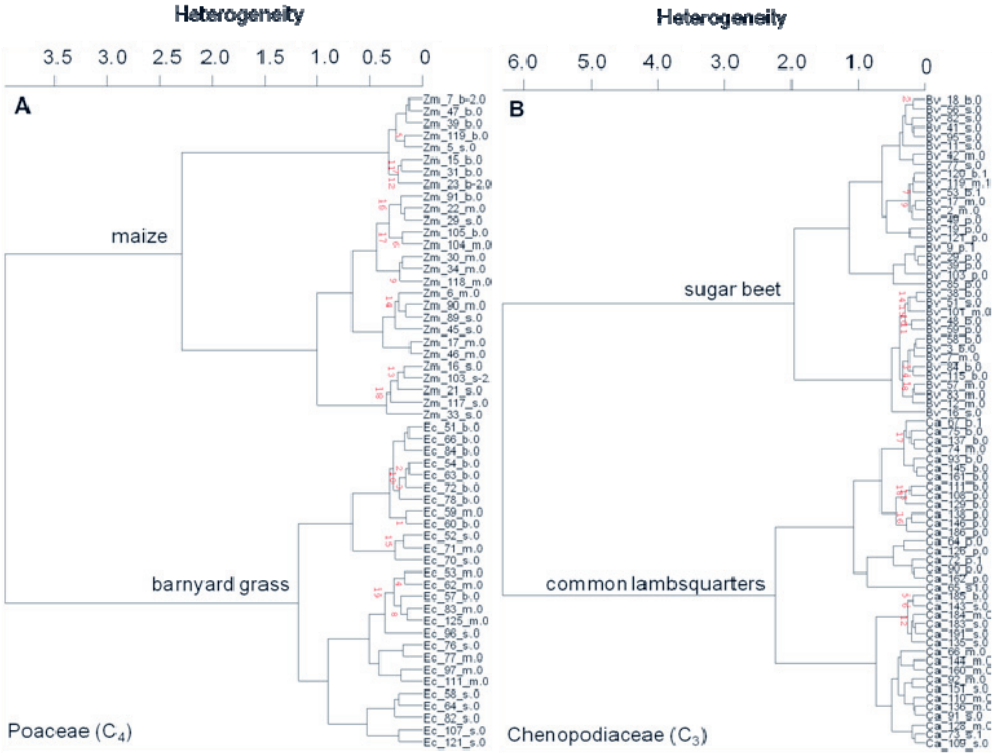

Fig. 2 Cluster analysis of FTIR-ATR spectra of maize, barnyard grass, sugar beet, and common lambsquarters. The first derivative was calculated and spectra were vector-normalized. Each spectra is labelled with species abbreviation (Zm=*Zea mays*, Ec=*Echinochloa crus-galli,* Bv=*Beta vulgaris*, Ca=*Chenopodium album*), sample number, and selected root section (b=basis, m=middle, s=tip, p=taproot). Cluster analyses are calculated of three (root basis, middle, and tip section) and four (plus taproot) root segments of nine individual per species. The spectral range from 3750-2750 cm^{-1} and 1800-600 cm^{-1} was considered.

Abb. 2 *Clusteranalyse der FTIR-ATR-Spektren von Mais- und Hühnerhirsewurzeln (A), sowie Zuckerrübe- und Weißer Gänsefußwurzeln (B). Die Daten wurden mit einer Vektornormierung und der ersten Ableitung vorbehandelt. Jedes Spektrum ist mit der Artabkürzung (Zm=Mais, Zea mays; Ec=Gemeine Hühnerhirse, Echinochloa crus-galli; Bv=Zuckerrübe, Beta vulgaris; Ca=Weißer Gänsefuß, Chenopodium album), Probennummer und dem Wurzelabschnitt (b=Basis, m=Mitte, s=Spitze, p=Pfahlwurzel) gekennzeichnet. In der Clusteranalyse sind Wurzelsegmente von neun Individuen und der spektrale Bereich von 3750-2750 cm^{-1} sowie 1800-600 cm^{-1} berücksichtigt.*

Cluster analyses of dry root spectra revealed a grouping of rootlets of the same segment. The maize and barnyard grass cluster showed subcluster of rootlets from the root basis (labelled b). Root

segments collected at root basis and taproots (labelled p) accumulated in the cluster of common lambsquarters.

Fresh roots of maize and barnyard grass showed avery similar spectra with marginal differences in peak height. Cluster analysis revealed no clear separation of root spectra according to species. Spectra of sugar beet and common lambsquarters also demonstrated very similar peak location but differed in peak height. Spectra of sugar beet had higher absorption rates in the range of 4000-600 cm^{-1} than those of barnyard grass. A complete separation of root spectra according to species was possible with taproots but not with other segments.

4. Discussion

4.1 Root discrimination by cluster analyses

FITR-ATR spectra represent the chemical composition of a sample. Corresponding to the substance, the mid-infrared radiation is absorbed and a sample-specific spectrum is recorded (GÜNZLER and GREMLICH, 2002) which can be used as a spectral fingerprint for sample identification. FTIR spectroscopy was already used for species discrimination of bacteria and fungi (NAUMANN et al. 1991, 2005; MARIEY et al. 2001; NAUMANN 2009). The results of this study show that dry rootlets of closely related species differ in FTIR-ATR spectra. Cluster analyses of FTIR-ATR spectra reveal a complete separation of rootlets according to species. A 100 % correct discrimination of peas and oat roots by cluster analysis of FTIR-ATR spectra was demonstrated by NAUMANN et al. (2010). In the cluster analysis, pea root spectra joined at 7.1 and oat root spectra at 2.9, while inter-specific heterogeneity was 25.2. Therefore, inter-specific heterogeneity was significantly higher than intra-specific heterogeneity. Pea and oat root spectra showed distinct differences in protein related peaks which were more pronounced in peas. Hence, the higher nitrogen and protein content in above-ground biomass was also reflected in the roots. In our study, we can exclude plant family-specific differences in spectral patterns. However, the FTIR-ATR spectra of maize, barnyard grass, sugar beets and common lambsquarters show differences in peak distribution and height. There is evidence that spectral patterns of FTIR-ATR spectra display species-specific attributes. A study by ZHAO et al. (2004) used the labour-intensive KBr pellet technique to record FTIR spectra of wheat and they found out that even wheat varieties differ in spectral pattern.

The spectra of dry rootlets in our study showed more pronounced species-specific differences than inter-specific differences or plant family-specific attributes. Therefore, we could demonstrate a species-specific root discrimination by cluster analysis. Fresh root spectra of the tested species did not differ significantly in peak distribution or height and could not be completely discriminated by cluster analysis. One reason for the similarity of the spectra could be the interference by water. Water is a strong absorbing substance which can cover peaks of other components in samples. Thus, it is recommended to dry the sample before recording the FTIR-ATR spectra (HSU, 1997).

Inter-specific competition between species that grew in shared pots showed neither significant effects on spectral patterns of dry roots nor on species separation by cluster analyses. This result supports the potential of FTIR-ATR spectroscopy and its appliance in further studies to test below-ground competition between crop and weed species or in intercropping systems. FTIR-ATR spectroscopy can help to overcome the difficulty to discriminate roots of herbaceous species and should be tested with more plant species. Furthermore, the results of the present study provide the basis of species quantification in root biomass which could be used for a deeper understanding of below-ground competition processes. LEI and BAUHUS (2010) developed a model for quantification of species ratio in root biomass. Species proportion in total root biomass was determined and quantified with near-infrared spectroscopy. The generated model successfully identified species proportion in root biomass of two, three, and four tree species plus herb roots. Models for quantification are also possible with FTIR-ATR spectroscopy. One promising approach for FTIR-ATR spectroscopy could be root biomass determination via soil cores and subsequent analysis of FTIR-ATR spectra to quantify species ratio of crop and weed species. The basic principle of the quantification model is the calibration with defined artificial root mixtures with involved species. First unpublished results

showed a sound model which demonstrated good approximation of true species ratios in the validation sample.

FTIR-ATR spectroscopy showed high potential for root discrimination of closely related species of one plant family. Further studies should test the method with field material and extended species range of crop and weed species to allow a detailed view in below-ground competition processes.

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