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Root Growth of Neighboring Maize and Weeds Studied with Minirhizotrons

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Competition between crops and weeds may be stronger at the root than at the shoot level, but belowground competition remains poorly understood, due to the lack of suitable methods for root discrimination. Using a transgenic maize line expressing green fluorescent protein (GFP), we nondestructively discriminated maize roots from weed roots. Interactions between GFP-expressing maize, common lambsquarters, and redroot pigweed were studied in two different experiments with plants arranged in rows at a higher plant density (using boxes with a surface area of 0.09 m^2) and in single-plant arrangements (using boxes with a surface area of 0.48 m^2). Root density was screened using minirhizotrons. Relative to maize that was grown alone, maize root density was reduced from 41 to 87% when it was grown with redroot pigweed and from 27 to 73% when it was grown with common lambsquarters compared to maize grown alone. The calculated root : shoot ratios as well as the results of shoot dry weight and root density showed that both weed species restricted root growth more than they restricted shoot growth of maize. The effect of maize on the root density of the weeds ranged from a reduction of 25% to an increase of 23% for common lambsquarters and a reduction of 42 to 6% for redroot pigweed. This study constitutes the first direct quantification of root growth and distribution of maize growing together with weeds. Here we demonstrate that the innovative use of transgenic GFP-expressing maize combined with the minirhizotron technique offers new insights on the nature of the response of major crops to belowground competition with weeds.

Nomenclature: Common lambsquarters, *Chenopodium album* L.; redroot pigweed, *Amaranthus retroflexus* L.; maize, *Zea mays* L.

Key words: Belowground competition, GFP, green fluorescent protein, lambsquarters, redroot pigweed, root density, weeds.

Aboveground interactions between weed and crops consist essentially of light competition, whereas belowground interactions involve competition for several resources, such as water and mineral nutrients, and the exudation of phytochemicals (Mahall and Callaway 1991; Schenk et al. 1999). The occupation and exploitation of the soil volume by the roots is critical for plant growth. Occupation results from the formation of lateral roots and the elongation of root axes, and it depends on assimilate allocation and root orientation, which determines the architecture of the root system. The arrangement of the roots in the soil ultimately determines the interactions among coexisting plants and the ability of plants to access soil resources. Resource exploitation also depends on root morphology and function (Casper and Jackson 1997).

Information on root interactions is essential for understanding the ecology of crop–weed communities. A number of studies have shown that the size of root systems increases when different wild or cultivated plant species grow in the same area (Falik et al. 2003; Gersani et al. 2001; Maina et al. 2002). These responses reflect a shift in assimilate partitioning, which suggests that soil resources, rather than light, are the most limiting factors in mixed-plant stands (Bloom et al. 1985; Hilbert 1990). The ability of plants to discriminate between their own roots and the roots of other plants has been proposed as the mechanism that underlies spatial segregation between root systems (Brisson and Reynolds 1994; Caldwell et al. 1991, 1996; Schenk et al. 1999). Root discrimination may also explain the simultaneous maximization of the competition with roots from other plants and minimization of the competition among the roots of an individual plant (Falik et al. 2003; Gersani et al. 2001). Until now, however, these hypotheses could not be rigorously tested because the direct measurement of root parameters in mixed-plant stands was impossible (Faget et al., 2013). Recently, Rewald et al. (2012) reviewed a diverse set of destructive strategies that have been used to identify roots of two or more species. These include (1) excavating the soil and tracing roots back to the plant to which they belong (Pechackova et al. 1999), (2) separating rooting volumes by barriers (Li et al. 2006) or trenches (Ludwig et al. 2004), (3) measuring the relative uptake of tracers such as ^{15}N (Jumpponen et al. 2002), (4) labeling the shoots of each species with different radioactive tracers to distinguish their roots from the differential signature on autoradiographs (Baldwin and Tinker 1972), (5) mapping all of the roots at the cut surface of a core and distinguishing roots by means of biochemical markers (Caldwell et al. 1996); and (6) using molecular tools based on the extraction of DNA and microsatellite analysis of root fragments (Mommer et al. 2008). Additionally, new approaches based on magnetic resonance imaging (Rascher et al. 2011) and the combination of this technique with position emission tomography (Jahnke et al. 2009) were developed to distinguish roots of two species in a nondestructive way.

These latter approaches responded to the need to identify roots from different plants throughout space and time and have been used with wild species or cultivated species such as beans and maize. A completely novel approach was recently developed by Faget et al. (2009) for the investigation of root dynamics: the combination of transgenic plants that express green fluorescent protein (GFP) with minirhizotrons, which are transparent tubes. This method allows the discrimination of the transgenic plant's roots from those of any other plant and thus enables the study of the spatial distribution of the roots of maize growing with weeds. The C_3 species common lambsquarters and the C_4 species redroot pigweed are noxious weeds affecting agronomic crops worldwide (Holm et al. 1997). Their vigorous early growth and prolific seed production allow them to compete aggressively with crops for light, water, and nutrients (Murphy et al. 1996), which results in the reduction of grain yield and quality (Knezevic et al. 1994). Because common lambsquarters germinates at

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lower temperatures than many other weed species (Weaver et al. 1988), it is particularly problematic for the cultivation of maize in temperate regions, where the crop is sown relatively late and exhibits slow early growth (Miedema 1982). Common lambsquarters is resilient to changes in the environment (Williams 1963) and to plant density (Rohrig and Stutzel 2001). According to Qasem (1993), common lambsquarters exhibits a high nutrient uptake because its roots can branch and elongate rapidly. The majority of studies that address the impact of common lambsquarters and redroot pigweed on maize growth have focused on aboveground factors, including the effect of weed density (Frantik 1994; Sheibany et al. 2009) and the relative emergence time of weed and crop plants (Baghestani and Aghabeigi 2006; Knezevic et al. 1994). Conversely, little is known about the impact of these two weeds on the root growth of maize.

Transgenic GFP-expressing maize and the weeds redroot pigweed and common lambsquarters were grown together or individually to determine whether the use of transgenic maize expressing GFP and the evaluation of root growth using minirhizotrons is a suitable approach to study root interactions between crops and weeds and to test whether the suitability of this approach depends on the weed species.

Materials and Methods

Experimental Conditions. The maize (genotype ETH-M72$_{\mathrm{GFP}}$), which expressed GFP (Aulinger et al. 2003), was grown together with common lambsquarters and redroot pigweed. The weed seeds were provided by the Agroscope Reckenholz-Tänikon Research Station, Zurich, Switzerland. The seeds were collected from wild populations in the years 1996 (redroot pigweed) and 2004 (common lambsquarters). In accordance with the legal restrictions on the use of genetically modified organisms in Switzerland, the experiment was conducted in a greenhouse, and the plants were harvested before the maize plants began to shed pollen. Containers were placed in greenhouse cabinets and were filled uniformly with the following sand-dominated commercial substrate (Ricoter A.G., Frauenfeld, Switzerland) to facilitate the root observations: 30 g kg^{-1} organic matter, 60 g kg^{-1} clay, 110 g kg^{-1} silt, 810 g kg^{-1} sand, pH 7.7, 79.9 mg kg^{-1} K, 4.1 mg kg^{-1} P, and 12 mg kg^{-1} N. Seeds of all plant species were pregerminated in trays of 0.30 (length) by 0.20 (width) by 0.05 m (height) that were filled with the same substrate as described above at 25 C. Upon germination, uniform seedlings were selected for each plant species and planted the same day. Reliable GFP expression of each ETH-M72$_{\mathrm{GFP}}$ maize seedling was verified before planting.

The environmental settings in the greenhouses were the same for all experiments: 25/21 C day/night temperatures, 50% relative humidity, and a photoperiod of 14 h light at 400 mol m^{2} s^{-1}. High-pressure mercury lamps (Philips HPL-N-400W; Royal Philips Electronics Inc., Amsterdam, Netherlands) were used as a complementary light source when the external light did not provide at least 25,000 lx. The greenhouses were built in 2007 and besides the sensor installed in the greenhouse we screened the environmental variables using Hobos (HOBO U12 Data Loggers, Onset Computer Corporation, Bourne, MA) placed at four additional locations. During the experiments, the plots were manually irrigated on a daily basis to provide a water column equivalent to 4 mm. Nitrogen in the form of ammonium nitrate (NH_4NO_3) was applied 16 d after planting to supply 40 g N m^{-2}.

Table 1. General description of the experiments.

Experiment abbreviation	R1	R2	P1	P2
Type of experiment[a]	R	R	P	P
Experiment run	1	2	1	2
Replications	4	6	6	6
Species arrangement	By row	By row	By plant	By plant
Number of plants per box	14	14	2	2
Duration in days	52	43	54	60
Time of the year	October–November 2008	April–May 2009	July–August 2009	October–November 2009
Treatments	Maize Redroot pigweed Common lambsquarters Maize + redroot pigweed Maize + common lambsquarters			

[a] R and P refer to row and position experiments, respectively.

The range of inferences that may validly be drawn from a study are principally determined by the experimental design used and thus the most suitable design is determined by the type of hypothesis to be tested (Freckleton and Watson 2000). Therefore, we evaluated the suitability of the GFP-based approach to study root interactions using two types of experimental arrangements commonly used to study interactions among plants (Table 1). We used two different types of boxes for each of these types of experiments (Figure 1). The two types of experiments were designed to generate conditions of plant competition where the GFP-based approach could be evaluated. In order to achieve this, we designed row experiments (R) based on a species ratio that can be found in additive and replacement designs where the density of a weed is higher than that of the crop and may also involve growing plants with and without interspecific competition (Park et al. 2003). The position experiments (P) were designed based on simple pair-wise mixtures where a 1 : 1 ratio of two competitors is maintained (Gibson et al. 1999). The P experiments were included to evaluate the GFP-based approach without confounding the effects of intra- and interspecific interactions and of species density and proportion. An additional goal of the plant arrangements that were used in both types of experiments was to evaluate if the GFP-based approach could provide information about the effects of plant interactions on root growth at different distances from the maize planting position, since these data may be highly relevant with neighborhood designs (Park et al. 2003).

R Experiments. Two minirhizotrons (56 mm internal diameter) were installed horizontally at a depth of 0.12 m (according to the uppermost side of the minirhizotron) in boxes measuring 0.40 (length) by 0.60 (width) by 0.40 m (height) (Figure 1a). The boxes were placed closed together in the glasshouse and at both ends of each row of boxes there was one additional box to reduce border effects. Four GFP maize plants were transplanted into a single row in the center of the plot and were spaced 0.10 m apart. A single row of weed plants was established on each side of the maize row with five plants in both rows. The distance between the plant rows was

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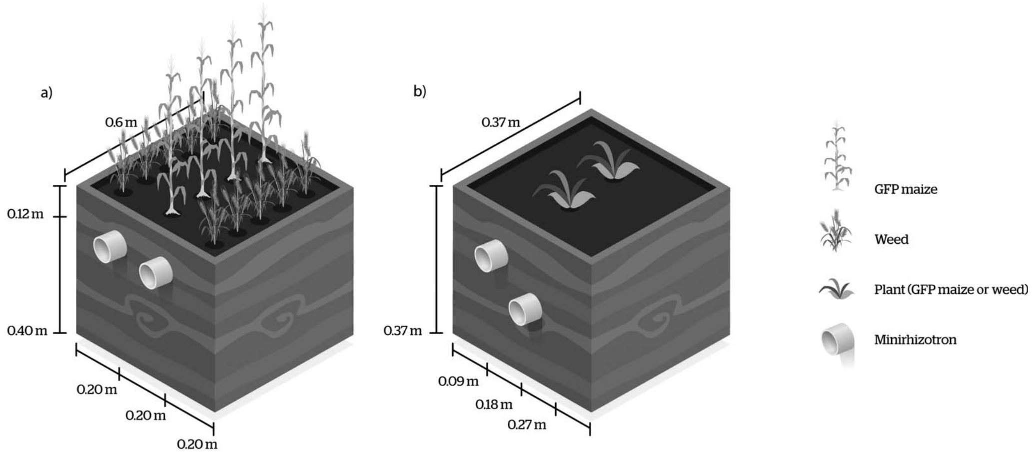

Figure 1. Schematic diagram of the boxes and plant arrangements that were used in (a) row experiments (R) and (b) position experiments (P).

0.20 m. The minirhizotrons were oriented parallel to the plant rows and placed between the two adjacent plant rows. One treatment (Table 1) was assigned per box and each of the boxes of the same replicate were arranged on the floor and distributed randomly within four (R1) and six (R2) areas inside one greenhouse cabinet. Each of the areas inside the glasshouse cabinet represented one block. The control boxes contained only one species, but the plants arrangement was identical to the arrangement of the mixed-species treatments. The experiment was conducted twice in a completely randomized layout. The first and second runs of this experiment are referred to as R1 and R2; R1 included four replicates and R2 included six replicates. R1 and R2 were conducted from October 7 to November 28, 2008 (52 d) and from March 30 to May 11, 2009 (43 d), respectively. When the experiments were terminated and the plants were harvested, the maize plants contained four to five fully developed leaves.

P Experiments. Two minirhizotrons (56 mm internal diameter) were installed horizontally at soil depths of 0.10 m and 0.20 m in boxes measuring 0.37 (length) by 0.27 (width) by 0.37 m (height) (Figure 1b). One treatment (Table 1) was assigned per box and each of the boxes of the same replicate were arranged on benches and distributed randomly within six areas inside a greenhouse cabinet. Each of the areas inside the glasshouse cabinet represented one block. The boxes were placed closed together in the glasshouse and at both ends of each row of boxes there was one additional box to reduce border effects. In these containers, single seedlings were planted at two positions that were 0.15 m apart on the central transect that was parallel to the long side. The P experiments included treatments in which both positions were occupied by plants in each box (see treatments in Table 1). The minirhizotrons were oriented parallel to the transect on which the plants were grown. The P experiments were conducted twice with six replicates. The experiments were conducted from July 7 to August 28, 2009 (P1, 54 d), and from October 20 to December 17, 2009 (P2, 60 d). When the experiments were terminated and the plants were harvested, the maize plants contained 8 to 10 (P1), or 10 to 12 (P2) fully developed leaves.

Sampling and Screening of Data. At harvest, shoots were cut at the ground level, dried at 80 C for 48 h, and weighed to determine their dry weight. Shoot dry weight is presented as the mean of four (R) and one (P) maize plants and 10 (R) and one (P) weed plants. Root images (19.5 by 26.0 mm) were recorded at the upper minirhizotron–soil interface using two camera configurations to distinguish the maize and weed roots. One camera configuration was used for sampling images that displayed all structures at the minirhizotron–soil interface, and the second camera configuration allowed for the precise identification of the ETH-M72$_{\text{GFP}}$ roots based on the GFP-emitted fluorescence (Faget et al. 2009). Minirhizotron images were recorded from the time of sowing until just before the plots were harvested. The data that are presented herein were derived from 12 (P) or 20 (R) images per minirhizotron and covered an area of 6,084 (P) and 10,140 (R) mm^2 per soil depth. Therefore, we evaluated the GFP-based approach covering a greater area per soil depth than the 100 mm^2 in most published studies (e.g., Thorup-Kristensen et al. 2006). Root counting was performed according to the methodology that was proposed by Upchurch and Ritchie (1983). If a root branched while intersecting the tube, it received one count for the main root and one for each branch. This value was converted into a surface unit (cm^2) and is hereafter referred to as the root density (roots cm^{-2}). The ratio of root density to shoot dry weight was calculated separately for the maize and weed plants by dividing the observed root density on a single minirhizotron by the total dry weight of all the shoots of each plant species.

Analysis. All data were analyzed independently for each run and checked for heteroscedasticity using Cook-Weisberg tests and by plotting residuals against fitted values for different candidate models. Influence of single observations and detection of outliers was evaluated by means of Cook's distance. Effects of autocorrelation were evaluated with graphs of residues and comparing models with different autocorrelation structure.

The plant dry weights and ratios of root density to shoot dry weight were analyzed using generalized least squares (GLS) as implemented in the R (R Development Core Team, 2007) package nlme (Pinheiro and Bates 2009). The former parameters were estimated using GLS due to evidence of heteroscedasticity. Model selection was performed with the R function "stepAIC" from the package MASS (Venables and

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Table 2. Dry weight of maize shoots in the presence of redroot pigweed and common lambsquarters in the first (R1) and second (R2) runs of the experiments in which the plant species were arranged by row and in the first (P1) and second (P2) runs of the experiments in which the plant species were arranged by position.

Experiment	R1	R2	P1	P2
T[a]	0.018	0.058	NS	NS
S	NS	NS	NS	NS
T × S	NS	NS	NS	NS
	g plant^{-1}			
Maize	4.31a[b]	3.11	7.40	7.45
Maize + redroot pigweed	2.99b	1.59	7.55	9.20
Maize + common lambsquarters	1.49b	2.13	7.25	8.40

[a] Abbreviations: T, treatment; S, weed species; NS, not significant.

[b] Values in the same column followed by different letters are significantly different from each other at the 95% probability level.

Ripley 2002). The full model included different sets of factors, depending on whether maize plants or weed plants were analyzed. For maize, a single effect (treatments) was modeled with the following levels: no weed competition, weed competition with redroot pigweed, and weed competition with common lambsquarters. For the weed plants, the effects of the plant species (redroot pigweed or common lambsquarters) and the presence of maize (with or without) were tested. In order to handle correlation among repeated measurements (plants within plots for the shoot dry weight and tubes within plots for the ratio of root density to shoot dry weight), autocorrelation was modeled as an autocorrelation structure of lag 1 with the "correlation" argument of the function "gls," whereas heteroscedasticity was handled by modeling the error variance as an exponential function with the "weights" argument of the same function.

The statistical analysis of the root data was conducted within the framework of general estimating equations (GEE) implemented in the R package geepack (Halekoh et al. 2006; Yan and Fine 2004). A GEE approach was chosen to account for autocorrelation among the adjacent images of a single minirhizotron tube, which were modeled as an autoregressive process of lag 1. Since root density was count data, it was fitted according to a Poisson distribution of the errors and using logarithm as the link function. Minirhizotron-within-plot was set as the clustering unit. For root data, the models used for the R experiments included the same effects as those that were described above for the shoot data, whereas the P experiments included soil depth as an additional factor. The model selection was based on Wald tests that were implemented in the function "anova.geeglm".

Results and Discussion

Effects of Weeds on Maize Shoot Growth. The maize shoot dry weight was marginally ($P < 0.10$) reduced by the presence of weeds in experiments R1 and R2 (Table 2). In R1 and R2, the dry weight of maize that was grown alone exceeded on average that of maize that was grown with redroot pigweed or common lambsquarters by 47 to 128%, respectively. In experiments P1, and P2, the differences between maize grown alone and maize grown with weeds were relatively small; they ranged between −2 and +23%.

Effects of Weeds on Maize Root Growth. The root density of maize was significantly ($P < 0.05$) reduced by the presence of both weed species in experiment R1 and at both soil depths in the P experiments. In experiment R2 the root density of maize was marginally reduced by the presence of weeds. In addition, in experiment P1, the decrease in the root density of maize that was grown together with common lambsquarters at the 0.10-m soil depth was insignificant compared to the root density of the maize that was grown alone (Table 3). Overall, the maize root density decreased by −41 to −87% and −26 to −73% for redroot pigweed and common lambsquarters, respectively. On average and based on all of the experiments, both weeds reduced the maize root density by over 45% at 0.10 m and 76% at 0.20 m. Therefore, the reduction in root density was more severe at the deeper soil depths.

Table 3. Root density of maize in the presence or absence of redroot pigweed and common lambsquarters at different soil depths. The data are from the first (R1) and second (R2) runs of the experiments in which the plant species were arranged by row and the first (P1) and second (P2) runs of the experiments in which the plants were arranged by position.

Experiment	R1	R2	P1	P2
	P-value			
Effects				
T	0.004	0.050	< 0.001	< 0.001
D	NA	NA	0.006	NS
T × D	NA	NA	0.002	NS
	roots cm^{-2}			
D = 0.10 m				
Maize	0.33a[b]	0.43a	2.56a	1.39a
Maize + redroot pigweed	0.19b	0.22b	1.51b	0.66b
Maize + common lambsquarters	0.15b	0.26b	1.88ab	0.55b
D = 0.20 m				
Maize	NA	NA	2.31a	1.35a
Maize + redroot pigweed	NA	NA	0.3c	0.32b
Maize + common lambsquarters	NA	NA	0.62b	0.41b

[a] Abbreviations:T, treatment; D, soil depth; NA, not available; NS, not significant.

[b] Values in the same column followed by different letters are significantly different from each other at the 95% probability level.

Soil depth significantly affected the maize root density in one of the two P experiments (P1). In experiment P1, the response was marginally modified by a soil depth by treatment interaction effect. In experiment P2, the root density of maize that was grown alone was similar at both soil depths. In experiment P1, the root density of maize was greater at 0.10 m than at 0.20 m, and this effect was stronger for maize grown with weeds than for maize grown alone. Within the treatments with weeds, the effect was stronger for maize that was grown with redroot pigweed than with common lambsquarters. Maize roots were more concentrated at the shallower soil depth in the presence of weeds than in the absence of them (experiments P1 and P2). We refer to the differences in root density at 0.20 and 0.10 m as "root stratification" (i.e., a higher root density at a certain soil depth as a consequence of the experimental treatment). Stronger root stratification was observed in the presence of redroot pigweed (two- to fivefold difference) than with common lambsquarters (1.3- to 3.0-fold difference). The lack of root stratification between 0.10 and 0.20 m when maize grew alone indicates that changes in the distribution of maize roots occurs in response to interspecific competition. In a ranked order, the maize root stratification was 1.03 (maize alone in experiment P2), 1.11 (maize alone in experiment P1), 1.34 (maize grown with common lambsquarters in experiment P2), 2.06 (maize grown with redroot pigweed in experiment P2), 3.03 (maize grown with common lambsquarters in experiment P1), and 5.00 (maize grown with redroot pigweed in experiment P1).

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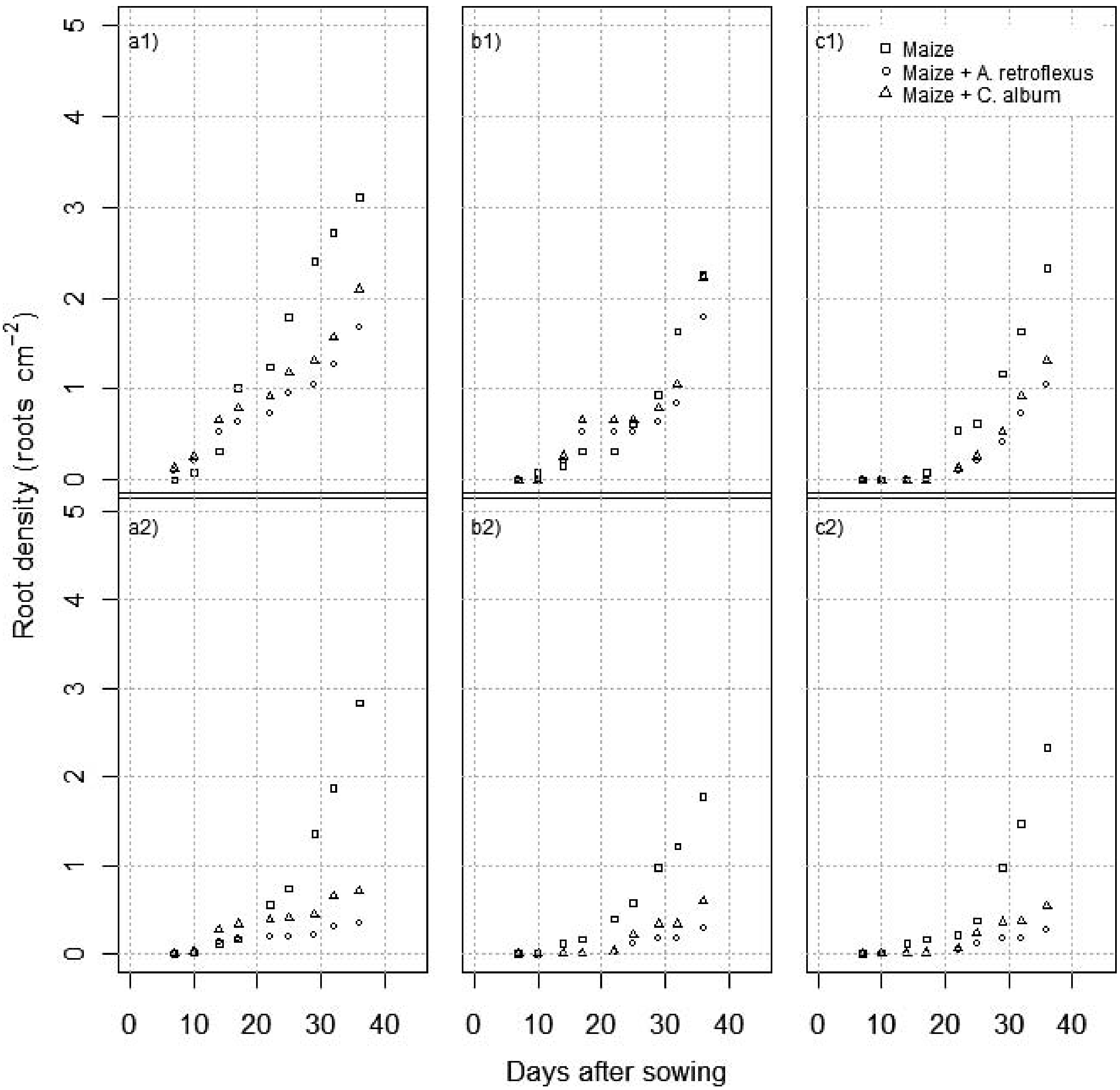

Figure 2. Example of root density dynamics of maize alone and influenced by the presence of weeds at different soil depths (1 = 0.10 m and 2 = 0.20 m) and different distances from the maize planting position (a = 0 to 78 mm, b = 79 to 156 mm, and c = 157 to 234 mm). Data are from experiment P1.

Figure 2 shows the root density of maize that was grown alone and the root density of maize in the presence of weeds in experiment P1. The results on root dynamics are an example of how this method can be used to assess the effects of weeds on the root growth of a main crop throughout the growing season at different soil depths and distances from the sowing position of the crop. In experiment P1, the root density of maize grown alone increased until the harvest of the experiment. The root density of maize with weeds was generally lower than that of maize alone throughout the growing season. In maize grown with weeds, a lag phase for the root growth compared to maize grown alone was observed at the two soil depths but increased markedly at 0.20 m and at increasing distances from the maize planting position. Our results were obtained with minirhizotrons, a method that minimizes interference with root growth (Taylor et. al. 1990). Minirhizotrons permit the simultaneous measurement of fine root production and disappearance, which cannot be accomplished using coring, in-growth cores, or excavation approaches. We did not observe ETH-M72$_{GFP}$ maize roots that lacked the fluorescent intensity associated with the GFP expression (Faget et al. 2009), allowing roots of ETH-M72$_{GFP}$ maize to be unequivocally distinguished from the roots of the weeds.

The negative influence of redroot pigweed (e.g., Frantik 1994; Knezevic et al. 1994; Sheibany et al. 2009; Vengris et al. 1953) and common lambsquarters (Fischer et al. 2004;

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Table 4. Ratio of root density to shoot dry weight of maize at different soil depths and in the presence or absence of redroot pigweed and common lambsquarters. The data are from the first (R1) run of the experiments in which the plant species were arranged by row and the first (P1) and second (P2) runs of the experiments in which the plants were arranged by position.

Experiment	R1	R2	P1	P2
	P-value			
Effects				
T	0.092	0.121	0.021	0.027
D	NA	NA	0.003	NS
T × D	NA	NA	0.017	NS
	roots cm^{-2} g^{-1} × 10^{-2}			
D = 0.10 m				
Maize	7.65	13.82	34.59a[b]	18.65a
Maize + redroot pigweed	6.35	13.84	20.00b	7.17b
Maize + common lambsquarters	10.07	12.21	25.93b	6.55b
D = 0.20 m				
Maize	NA	NA	31.21a	18.12a
Maize + redroot pigweed	NA	NA	3.97b	3.48b
Maize + common lambsquarters	NA	NA	8.55b	4.88b

[a] Abbreviations: T, treatment; D, soil depth; NA, not available; NS, not significant.

[b] Values in the same column followed by different letters are significantly different from each other at the 95% probability level.

Table 5. Shoot dry weight of redroot pigweed and common lambsquarters in the presence or absence of maize. The data are from the first (R1) and second (R2) runs of the experiments in which the plant species were arranged by row and the first (P1) and second (P2) runs of the experiments in which the plants were arranged by position.

Experiment	R1	R2	P1	P2
	P-value			
S[a]	< 0.001	0.067	0.020	0.010
T	0.001	NS	0.009	0.017
T × S	0.003	NS	NS	NS
	g $plant^{-1}$			
Redroot pigweed + maize	2.27b[b]	3.44a	13.60a	5.57a
Common lambsquarters + maize	1.88bc	2.12b	9.45b	3.29b
Redroot pigweed	3.36a	3.92a	11.24ab	7.23a
Common lambsquarters	1.72c	3.29a	7.08c	3.09b

[a] Abbreviations: S, weed species; T, treatment; NS, not significant.

[b] Values in the same column followed by different letters are significantly different from each other at the 95% probability level.

Weaver 2001) on maize growth are well documented. Several trends were observed with respect to the effects on maize shoots and roots: (1) both were equally affected (with redroot pigweed in R2), (2) the shoot was more affected (with common lambsquarters in R1), or (3) the roots were more affected (with redroot pigweed in R1 and with both redroot pigweed and with common lambsquarters in experiments P1 and P2). Therefore, based on the number of experiments that showed a significant effect of the weeds on maize growth, the root density of maize was clearly more affected than the shoot growth. Averaged across all experiments, the effect on root density was double the effect on shoot growth.

The growth of maize was generally reduced already at early stages by the presence of weeds (e.g., Figure 2). This effect may have implications for weed control, which is typically implemented during the early developmental stages of maize growth. The water and nutrient uptake capacity of maize is already hampered at the establishment phase because of its relatively small root system (Liedgens and Richner 2001). In later stages, and especially after canopy closure, the competitive ability of maize against most weeds increases because the weeds are increasingly shaded (Hall et al. 1992; Swanton et al. 2000). As a consequence of reduced root growth, maize may experience reduced access to water and nutrients. One management option that can compensate for this nitrogen-access reduction is to increase nitrogen fertilization, which can increase maize productivity but has detrimental environmental effects (Garibay et al. 1997). Therefore, methods that allow for studying root dynamics are necessary to identify stages when root growth may limit resource uptake.

Effects of Weeds on Root : Shoot (R : S) Ratio of Maize. R : S ratios are useful to understand the physiological basis of species' interactions (Bi and Turvey 1994). There was a tendency ($P < 0.1$) for weeds and soil depth to influence the ratio of the root density of maize to the shoot dry weight (R : S ratio). Except in experiment R1, the R : S ratio was higher for maize grown alone than for maize grown with weeds. The ranking of the R : S ratio of maize grown with weeds varied across experiments and the differences between them were not significant (Table 4).

By including monospecific treatments (i.e., treatments in which only maize or the weeds were grown), interesting aspects of intraspecific competition became evident. The shoot mass was similar among the treatments including weeds and maize alone. These results are in line with the general conclusions of Baghestani and Aghabeigi (2006) and Murphy et al. (1996), who reported that due to intensive selection, crop plants have a high nutrient uptake capacity, which corresponds to high competitive ability. Thus, due to the high competitive ability, maize plants affect each other as strongly as they are affected by weed plants.

Effects of Maize on Shoot Growth of Weeds. With or without maize competition, redroot pigweed produced significantly larger shoots than common lambsquarters in experiments R1, P1, and P2 (Table 5). The same effect, albeit marginally significant, was found in experiment R2. The presence of maize always increased the differences between the two weed species. The effect of maize on the shoot dry weight of a weed species varied across the experiments; however, significant reduction in the shoot dry weight of the weeds was more the exception than the rule. This was found for redroot pigweed in experiment R1. In addition, a marginally significant reduction in the shoot dry weight of common lambsquarters was found in experiment R2. The opposite was found in experiment P1, in which the weed shoot weights increased when they were grown with maize plants, but it was significantly different only for common lambsquarters. The shoot weights of common lambsquarters grown with maize were greater than those that were grown alone (in experiments R1, P1, and P2), whereas the shoot weights of redroot pigweed were reduced by the presence of maize (except in experiment P1).

Effects of Maize on Root Growth of Weeds. The statistical analysis of the root density of the weeds indicated the presence of diverse results among the different experiments (Table 6). No significant differences were found between the root densities of the weeds in two experiments, P1 and P2. In the other two experiments, R1 and R2, redroot pigweed had significantly higher root densities than common lambsquarters. In experiment R1, there was a significant interaction

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Table 6. Root density of redroot pigweed and common lambsquarters in the presence or absence of maize at different soil depths. The data are from the first (R1) run of the experiments in which the plant species were arranged by row and the first (P1) and second (P2) runs of the experiments in which the plants were arranged by position.

Experiment	R1	R2	P1	P2
	P-value			
Effect				
S[a]	NS	0.042	NS	NS
P	NS	NS	0.0343	0.0630
D	NA	NA	NS	NS
S × P	0.0115	NS	NS	NS
S × D	NA	NA	0.0950	0.0810
P × D	NA	NA	NS	NS
S × P × D	NA	NA	NS	NS
	roots cm^{-2}			
D = 0.10 m				
Redroot pigweed + maize	0.92 ab[b]	0.53b	1.83 b	0.81 b
Common lambsquarters + maize	1.10 a	0.60b	1.85 b	0.91 ab
Redroot pigweed	1.09 a	0.91a	2.46 a	1.04 a
Common lambsquarters	0.86 b	0.62b	2.47 a	1.16 a
D = 0.20 m				
Redroot pigweed + maize	NA	NA	1.99 a	0.93 b
Common lambsquarters + maize	NA	NA	2.00 a	0.80 b
Redroot pigweed	NA	NA	2.11 a	1.19 a
Common lambsquarters	NA	NA	2.12 a	1.02 ab

[a] Abbreviations: S, weed species; P, presence of maize; D, soil depth; NS, not significant; NA, not available.

[b] Values in the same column followed by different letters are significantly different from each other at the 95% probability level.

Table 7. Ratio of root density to shoot dry weight of redroot pigweed and common lambsquarters in the presence or absence of maize at different soil depths. The data are from the first (R1) and second (R2) runs of the experiments in which the plant species were arranged by row and the first (P1) and second (P2) runs of the experiments in which the plants were arranged by position.

Experiment	R1	R2	P1	P2
	P-value			
Effect				
S[a]	0.0008	0.0029	< 0.0001	< 0.0001
P	0.0107	0.0175	0.0477	NS
D	NA	NA	0.0683	NS
S × P	NS	0.0013	NS	0.0676
S × D	NA	NA	0.0252	0.0990
P × D	NA	NA	NS	NS
S × P × D	NA	NA	NS	NS
	roots $\mathrm{cm}^{-2}\ \mathrm{g}^{-1} \times 10^{-2}$			
D = 0.10 m				
Redroot pigweed + maize	40.53b[b]	15.40b	13.45c	14.54c
Common lambsquarters + maize	58.51a	28.42a	19.58c	27.66b
Redroot pigweed	32.44c	23.31a	21.88b	14.38c
Common lambsquarters	50.00b	19.11b	34.89a	37.54a
D = 0.20 m				
Redroot pigweed + maize	NA	NA	14.63b	16.70c
Common lambsquarters + maize	NA	NA	21.16a	24.32b
Redroot pigweed	NA	NA	18.77b	16.46c
Common lambsquarters	NA	NA	29.94a	33.01a

[a] Abbreviations: S, weed species; P, presence of maize; D, soil depth; NS, not significant; NA, not available.

[b] Values in the same column followed by different letters are significantly different from each other at the 95% probability level.

between the weed species and the presence of maize; the root densities of common lambsquarters and redroot pigweed increased and decreased, respectively, in the presence of maize. This was the only experiment where such interaction was found. The presence of maize reduced significantly the root density of redroot pigweed at 0.10 m in experiment P1. A reduction in the root density of common lambsquarters due to the presence of maize was found only in experiment P1 at 0.10 m. To the best of our knowledge this is the first study that analyzed the root growth dynamics of common lambsquarters and redroot pigweed.

Effects of Maize on R : S Ratio of Weeds. Independent of the maize presence, the R : S ratio of the weeds was generally larger for common lambsquarters than for redroot pigweed (Table 7). The only exception to this general trend was observed in experiment R2, in which the R : S ratio of redroot pigweed was larger than that of common lambsquarters. Besides to these effects, the R : S ratio was marginally affected by soil depth in the P1 experiment. The R : S ratio was also affected by interactions with the weed species in experiments P1 and P2, although these interactions were marginally significant in experiment P2. In these two experiments, the R : S ratio of redroot pigweed in the presence of maize was greater at the 0.20-m than at the 0.10-m soil depth, whereas the R : S ratio of common lambsquarters in the presence of maize was greater at the 0.20-m soil depth in experiment P1 and not significantly different in experiment P2. It is important to note that in the absence of maize both weeds tended to have a higher R : S ratio at 0.10 m (Table 7). An effect on the R : S ratio of common lambsquarters was observed at 0.10 m and in only one experiment at 0.20 m (i.e., experiment P2) and no effect was found on the R : S ratio of redroot pigweed. When the corresponding response was observed in maize, it was found at both soil depths. At the 0.20-m depth, the effect on the R : S ratio was reversed (i.e., the ratio was greater for maize that was grown alone than for maize that was grown with weeds).

Compared Response of the Root System of Maize and Weeds. As in the case of maize, the presence of weeds had a greater effect on root density than on shoot weight. The only exception was experiment R1, where the major impact of the presence of maize was found for both weeds aboveground. The contrasting effects in weeds and maize can be interpreted as a maize response that alters its root distribution in the soil profile to favor shoot growth in response to the presence of weeds. In contrast, the weed response in the presence of maize is a more general change in the root system relative to the shoot. The results suggest that the root systems of the weeds included in this study established more efficiently and expanded into deeper soil layers than the maize root system in the presence of neighbors. The reaction of the weeds to the presence of maize is characterized by an increase in root density at the expense of shoot mass. There is evidence that maize grown under interspecific competition experiences root-distribution changes such that fewer roots develop in deeper soil layers. Maize in P experiments grew under interspecific and also intraspecific competition and whereas the root density of maize at 0.10 and 0.20 m was similar under intraespecific competition, root density at 0.20 m was on average 56% lower than at 0.10 m under interspecific competition (Table 3). Since the R : S ratio of maize was lower with interspecific than with intraspecific competition (Table 4), besides a reduced root growth due to shoot reduction, a reduction in root growth independent from the

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shoot occurred due to interspecific competition. The two weeds had a more consistent response above- and belowground to interspecific competition than did maize; the weeds exhibited a response that was characterized by an increase in root density at the expense of shoot mass.

Root Discrimination between Maize and Weeds. This study was made possible by an innovative approach that combined transgenic GFP-expressing maize with the minirhizotron technique. This method facilitates the observation of the root growth of one plant species that is growing in a mixed stand of plants. By discriminating between GFP-derived fluorescent and nonfluorescent roots (Faget et al. 2009), we were able to directly quantify in situ the relative proportion of maize and weed roots. It was possible to screen the dynamics of root growth in two experiments with different experimental settings in terms of design, plant density, and spatial arrangement of the plants. Maize and weeds that are grown under competition had contrasting effects. Maize that was grown in competition with common lambsquarters and redroot pigweed was more strongly impaired at the root than the shoot level, and these reductions were independent of the weed species.

Our results challenge the conclusions that were drawn in previous studies, which show that plants produce more roots when they are grown with another plant species than when they are grown alone (Callaway 2002; De Kroon et al. 2003; Gersani et al. 2001; Li et al. 2006; Mahall and Callaway 1991). Therefore, the response may depend on plant species and to the best of our knowledge there are no reports on maize with common lambsquarters and redroot pigweed. However, it is also important to note that most of these studies relied on split-root designs in which the roots of a plant are separated into two adjacent pots and allowed to intermingle with the roots of another plant in only one of the pots. Such methods dramatically disturb the soil environment and can lead to artifacts in plant growth (Connell 1990; McPhee and Aarseen 2001). The growth response is subsequently compared with that of a plant growing alone in the absence of the roots of another plant (Falik et al. 2003; Gersani et al. 2001).

According to Park et al. (2003), definitions of plant competition can be divided in two categories, those that focus on resource acquisition and those that focus on the reduction in fitness due to competition. The type of definition considered has an influence on the design of the experiment and the interpretation of its results. Therefore, the use of the GFP-based approach to study root interactions and a better understanding of belowground competition could be fundamental to reconcile results obtained with the previous two contrasting definitions of plant competition. Greenhouse experiments have been criticized on the grounds that the environment is too simplistic and has limitations to its ability to mimic the cumulative interacting effects that occur under the typical multivariate environment that determine plant interactions at the field level (Gibson et al. 1999). Slight variations in plant arrangement, soil volume, and greenhouse climate could have large impacts on the root growth and R : S ratios. It is especially important in studies on plant interactions to have representative light conditions, including light intensity and uniformity and to avoid unnatural ratios of red to far red light that may affect plant morphogenesis in an unrealistic fashion. The experiment was conducted in a greenhouse due to legal regulations that forbid using genetically modified plants outside. However, the findings of this study suggest useful applications of this method for field experiments. The GFP-based approach allowed for the first time the direct screening of root growth of competing weeds and maize. Therefore, the method used here paves the way for future studies of the aspects of belowground crop–weed interactions in agriculture. Findings related to the spatial distribution of maize roots interacting with weeds may further the understanding and management of crops (Schenk 2006). It may also be useful to apply the minirhizotron-based method presented here in combination with destructive (e.g., auger) sampling. This would allow the study of root dynamics and aspects related to root uptake and distribution for which destructive methods are more suitable.

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