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RESEARCH PAPER

Pseudomonas fluorescens CHA0 maintains carbon delivery to *Fusarium graminearum*-infected roots and prevents reduction in biomass of barley shoots through systemic interactions

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Abstract

Soil bacteria such as pseudomonads may reduce pathogen pressure for plants, both by activating plant defence mechanisms and by inhibiting pathogens directly due to the production of antibiotics. These effects are hard to distinguish under field conditions, impairing estimations of their relative contributions to plant health. A split-root system was set up with barley to quantify systemic and local effects of pre-inoculation with *Pseudomonas fluorescens* on the subsequent infection process by the fungal pathogen *Fusarium graminearum*. One root half was inoculated with *F. graminearum* in combination with *P. fluorescens* strain CHA0 or its isogenic antibiotic-deficient mutant CHA19. Bacteria were inoculated either together with the fungal pathogen or in separate halves of the root system to separate local and systemic effects. The short-term plant response to fungal infection was followed by using the short-lived isotopic tracer $^{11}CO_2$ to track the delivery of recent photoassimilates to each root half. In the absence of bacteria, fungal infection diverted carbon from the shoot to healthy roots, rather than to infected roots, although the overall partitioning from the shoot to the entire root system was not modified. Both local and systemic pre-inoculation with *P. fluorescens* CHA0 prevented the diversion of carbon as well as preventing a reduction in plant biomass in response to *F. graminearum* infection, whereas the non-antibiotic-producing mutant CHA19 lacked this ability. The results suggest that the activation of plant defences is a central feature of biocontrol bacteria which may even surpass the effects of direct pathogen inhibition.

Key words: Biocontrol, carbon partitioning, ^{11}C, *Fusarium graminearum*, *Hordeum vulgare*, induced systemic resistance (ISR), PGPR, *Pseudomonas fluorescens*, signaling.

Introduction

The necrotrophic fungus *Fusarium graminearum* (Schwabe) is a devastating pathogen of barley and other cereals, causing significant yield losses by diseases known as 'Fusarium head blight' and 'root rot' (Parry *et al.*, 1995) and also by contaminating the grains with fungal toxins (Mesterhazy, 2002). Plants use different mechanisms of constitutive and induced defence against microbial pathogens, comprising chitinase production or lignification of the cell wall (Kang and Buchenauer, 2000). Upon encounter, pattern recognition receptors (PRRs) in the plant cell membrane identify the type of pathogen and activate mitogen-acivated protein kinase (MAPK) cascade signalling pathways to direct plant resources for optimal investment of pathogen defence for local and systemic resistance (Zhang and Klessig, 2001).

Soil microorganisms are key regulators of plant resistance against pathogens, and beneficial bacteria can strongly influence the health status of plants. In particular, pseudomonads form a ubiquitous and well described group of plant beneficial bacteria that affect the potential of pathogens to infect plants. Pseudomonads synthesize a variety of antifungal compounds, which inhibit the growth of numerous soil-borne pathogens (Compant *et al.*, 2005) and prevent plant infection. Moreover, these bacteria elicit plant-induced systemic resistance (ISR) in which a plant reacts to metabolites and antigens of these bacteria. For example, the antifungal bacterial toxin 2,4-diacetylphloroglucinol (DAPG) elicits ISR (Iavicoli *et al.*, 2003). ISR arises from an up-regulation of pathogenesis-related (PR) genes (Bakker, 2007) which protects against further infections by fungal pathogens. Thus, both inhibition of pathogens and activation of plant defences reduce pathogen impact. However, these effects have usually been described separately (Pieterse *et al.*, 2003; Compant *et al.*, 2005), making it hard to evaluate their relative importance in plant health.

In this study the relative contribution of pathogen inhibition and elicitation of plant defences by *Pseudomonas fluorescens* CHA0 on the infection of barley plants by the soil-borne pathogen *F. graminearum* was investigated. The contributions of pathogen inhibition and elicitation of plant defences, each caused by *P. fluorescens*, were deduced by comparing its local and systemic effects on barley plants which were subsequently infected by the soil-borne pathogen *F. graminearum*. Plants rapidly respond to pathogen or herbivore attack by altering the partitioning of photoassimilates (Anten and Pierik, 2010), and diversion of carbon from infected roots is a marker for early recognition of pathogen attack (Henkes *et al.*, 2008). These changes are modulated by the jasmonic acid pathway (Henkes *et al.*, 2008), a pathway central for resistance against *F. graminearum* in wheat (Li and Yen, 2008).

In this study, the rapid changes in carbon partitioning were followed by application of $^{11}CO_2$ to a leaf and monitoring carbon allocation of the tracer in the plant in a non-invasive manner with high time resolution. In addition, the local and systemic long-term effects of pre-inoculation with *P. fluorescens* on plant shoot and root biomass in response to subsequent infection with *F. graminearum* were investigated in a split-root system.

Materials and methods

Plants

Seeds of barley (*Hordeum vulgare* L. cv. 'Barke', Irnich Inc., Frechen, Germany) were dehusked by incubation in 50% H_2SO_4 for 60 min under agitation, and washed three times with distilled water to remove the acid. Seeds were sterilized with a freshly prepared 2% $AgNO_3$ solution for 20 min on a shaker at 200 rpm, washed with a sterile 1% NaCl solution, with distilled water, again with NaCl, and five times with distilled water to remove the remaining $AgNO_3$ completely.

The seeds were germinated in darkness at 20 °C on a diluted nutrient agar [agar 8 g l^{-1}, nutrient broth 0.8 g l^{-1} in Neff's modified amoeba saline (AS; Page, 1988)]. After 4 d, seedlings were checked visually for contaminations with microorganisms under the microscope and in addition no clouding of growth medium was observed. Sterile plants were transferred into silicone closed-cell foam rubber stoppers with a longitudinal slit (VWR, Darmstadt, Germany) which then sealed the roots into glass tubes (length 135 mm, 25 mm diameter) containing 50 ml of sterile 50% Hoagland solution. The plants were grown at 60% relative humidity with 16 h daylength (light intensity of 100 µE m^{-2} s^{-1}, temperature 25°C) and 8 h night (20°C). After 7 d, individual plants were transferred into two-chamber split-root rhizotrons. The roots were separated into two roughly equal parts into the two chambers of the rhizotron, and each chamber was sealed with silicone grease (Baysilone, Bayer, Germany). The rhizotron chambers were each supplied with 300 ml of 50% Hoagland solution containing 5 mM MES buffer [2-(*N*-morpholino) ethanesulphonic acid; pH 5.8] and plants were allowed to grow for an additional 5–7 d until radiotracer experiments were started with 2- to 3-week-old plants.

Fusarium graminearum inoculum

The pathogenic fungus *F. graminearum* (Schwabe) strain DSM 1095, isolated from *Zea mays* roots, was obtained from 'Deutsche Sammlung von Mikroorganismen und Zellkulturen' (DSMZ; Braunschweig, Germany). The strain was kept on Luria–Bertani (LB) agar plates at 20 °C. To prepare inoculums for the infection of the roots, a piece of hyphal material of the *F. graminearum* culture was taken from the agar plate and grown in liquid LB medium for 2 d at 20 °C under agitation of 200 rpm, and adjusted to an OD_{600} of 0.45. The inoculum consisted exclusively of fungal hyphae, with no detectable macrospores. According to the treatment (see below), 5 ml of this inoculum was introduced to one or both sides of the split-root system.

Pseudomonas strains

Pseudomonas fluorescens CHA0 and its isogenic *gacS*-deficient mutant CHA19, carrying a mini Tn7 chromosomal green fluorescent protein (GFP) insert (Jousset *et al.*, 2006), were kept routinely on nutrient agar (blood agar base 40 g l^{-1}, yeast extract 5 g l^{-1}) supplemented with 25 µg ml^{-1} kanamycin (Sigma, Germany). Prior to inoculation, bacteria were grown in NYB medium (Nutrient Yeast Broth; nutrient broth 25 g l^{-1}, yeast extract 5 g l^{-1}) at 30 °C under agitation of 200 rpm. Late exponential phase bacteria were harvested by centrifugation (5300 *g* for 2 min), washed in saline solution (0.9% NaCl), and resuspended to an OD_{600} of 0.2 in AS medium which corresponds to a bacterial concentration of 0.4×10^9 colony-forming units (CFU) per ml.

Split-root rhizotrons

A split-root system in which pathogen and bacteria were applied either together or in separate compartments of the root system allowed the identification of either local or systemic effects.

Split-root rhizotrons (Fig. 1) for ^{11}C experiments were built from a single block of polypropylene (330 mm height, 235 mm width, 18 mm depth) with a transparent polycarbonate cover. Prior to each experiment, the rhizotrons were autoclaved for 20 min at 120 °C and 1.1 bar. Each side of the rhizotron was filled with 300 ml of a sterile hydroponic solution containing 50% Hoagland (buffered with 5 mM MES; adjusted to pH 5.8 with KOH). To avoid anoxia during the experiments, both root halves were aerated with sterile filtered air. Long-term effects of *Fusarium* infection on barley biomass were investigated using a similar protocol but with a simplified split-root system (see below).

^{11}C labelling

The effect of pathogen infection on a barley plant was quantified non-invasively by the use of ^{11}C to follow carbon partitioning, a very sensitive measure of whole-plant responses. The short 20 min half-life of the ^{11}C tracer permits repeated labelling of each plant to follow changes over the long term as well. Two to three days prior to an ^{11}C experiment a plant was transferred to acclimate in the growth chamber used for radiotracer studies. At 15 h prior to labelling, the second leaf was sealed with two-component silicone rubber (Xantopren VL, Heraeus Kulzer, Hanau, Germany) into a cylindrical Plexiglas™ leaf chamber (70 mm length, 18 mm diameter), and allowed to recover from the mechanical disturbance. The leaf chamber received air from outside and, then, at ~4 h into the photoperiod, it was connected to the CO_2- and humidity-regulated closed gas exchange system in preparation for labelling. The leaf was pulse-labelled three times per day on two sequential days, with ~100 MBq of $^{11}CO_2$ at ~5.0, 7.5, and 10.5 h into the photoperiod. Light intensity was 350 µE m^{-2} s^{-1} at the load leaf and 300 µE m^{-2} s^{-1} at the rest of the shoot. Fresh Hoagland solution was added as necessary through a 0.22 µm filter. The $^{11}CO_2$ was produced in the INC, Forschungszentrum Jülich.

Treatments

To investigate the effect of *F. graminearum* on the carbon partitioning between the two root halves, one side of the root system (Fus+) was inoculated with 5 ml of freshly prepared *F. graminearum* inoculum in LB medium in each of these experiments. The other side (Fus–) received an equal quantity of sterile LB medium. This experiment denoted 'Fus' was repeated eight times with different plants. In a second experiment, the entire root system was infected with 10 ml of *Fusarium* inoculum (denoted Fus/Fus; n=3 replicates with different plants).

To investigate whether *P. fluorescens* affected carbon partitioning, 3 ml of a suspension of CHA0 or CHA19 (OD_{600}=0.2) in AS medium were added to one side of the root system during the second ^{11}C pulse-labelling interval (see treatment timing below). The control side received 3 ml of sterile AS. Experiments were repeated nine times with separate plants with the strain CHA0, and 10 times with the *gacS* mutant CHA19.

To test for a local and systemic effect of *P. fluorescens* CHA0 upon subsequent infection by *F. graminearum*, one side of the root system was inoculated with one of the *P. fluorescens* strains as described above, and left for 48 h to allow the bacteria to colonize the roots. Then the plants were placed into the ^{11}C measuring system and infected with *F. graminearum* as described above, in the following combinations: (i) CHA0 and *F. graminearum* on the same root half to test the direct effect of *P. fluorescens* on subsequent infection by *F. graminearum* (Fus&CHA0; n=5); (ii) as a control, the non-antibiotic-producing mutant CHA19 and *F. graminearum* on the same root half (Fus&CHA19; n=6); and (iii) CHA0 and *F. graminearum* on different sides of the root system to test for a systemic effect of *P. fluorescens* on subsequent infection by *F. graminearum* (Fus/CHA0; n=5).

Treatment timing for ^{11}C experiments

For the fungal treatments, root portions were infected during the second pulse of ^{11}C labelling when tracer activity in the root detectors peaked (i.e. equal rates of decay and arrival), 60–70 min after the start of labelling. With this timing any immediate change in transport could be detected sensitively with a 60 s time resolution (Minchin and Thorpe, 2003). In contrast to the rapid effect of jasmonic acid (Henkes *et al.*, 2008), no fast changes in partitioning were observed here, and therefore just one value was calculated for each of the six ^{11}C pulses.

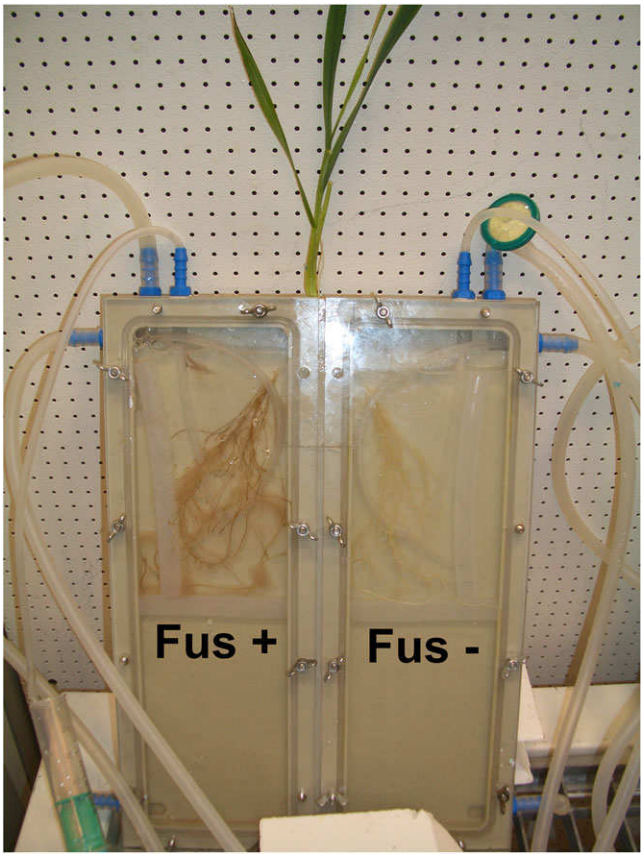

Fig. 1. Split-root rhizotron for infecting half of the barley root system with *Fusarium graminearum* (Fus+) while the other half is kept sterile (Fus–), showing a barley plant 4 d after infection by *F. graminearum*. Aeration was stopped while taking the photograph.

^{11}C detection and analysis

Scintillation detectors [Bicron NaI(Tl) detectors, Saint-Gobain Crystals, Houston, TX, USA] were positioned within radiation shielding to be uniformly sensitive to well-defined parts of the plant. Detector counts of the γ radiation arising from β+ annihilation were corrected for background, dead time, and their different sensitivities to equal amounts of tracer. Activity was measured independently for the following plant parts: (i) shoot (i.e. the complete shoot except the load leaf); (ii) left root; and (iii) right root. Strips of clear 4 mm thick Plexiglas™ were placed around the shoot of the plant, to ensure that β+ radiation escaping from the tissue was annihilated near its source. To analyse the ^{11}C tracer time series, the 'input–output' method (Minchin and Troughton, 1980) was used, which estimates the transfer function for movement of tracer along a specific transport pathway in the plant (Minchin and Thorpe, 2003). The pathway is defined by the input and the output flow of tracer for a specific plant part. The mobilized carbon from the leaf was considered as input, and tracer entering either of the root portions (or their sum) was considered as output. By accounting for radioisotope decay the analysis quantifies the

transport of 'recently assimilated carbon', with the steady-state gain of the transfer function equal to the partitioning of the mobilized photoassimilates into the respective sink tissue. Partitioning values were normalized by dividing each data set by its initial value, in order to facilitate comparison of treatment responses between plants, assuming that a response was proportional.

Effects of F. graminearum *and* P. fluorescens *infection on barley biomass*

To detect effects of *Fusarium* infection in combination with pre-inoculation of either one of the *P. fluorescens* strains on plant biomass, 7-day-old plant seedlings were placed in one microcosm each. Microcosms were constructed from 250×160×15 mm polycarbonate plates (Jousset *et al.*, 2011) and were autoclaved prior to establishing the seedlings. Each chamber of the microcosm (100×150×5 mm) was filled with 15 ml of an agarized (2% agar) and sterile 50% Hoagland's nutrient solution. After the microcosms had cooled, seedlings were transferred to the chambers and the roots of each plant were distributed equally into the two split-root chambers. The following treatment combinations were tested: (i) untreated barley plant (Ctrl; n=6); (ii) non-antibiotic-producing mutant *P. fluorescens* CHA19 and *F. graminearum* on the same root half to test the direct effect of *P. fluorescens* on biomass after subsequent infection by *F. graminearum* (Fus&CHA19; n=6); (iii) wild-type *P. fluorescens* CHA0 and *F. graminearum* on the same root half (Fus&CHA0; n=6); and (iv) CHA0 and *F. graminearum* on different halves of the root system, to test for a systemic effect of *P. fluorescens* on biomass after subsequent infection by *F. graminearum* (Fus/CHA0; n=6).

For the *Pseudomonas* pre-treatments, 1 ml of a *P. fluorescens* CHA0 or CHA19 suspension (adjusted to an OD_{600}=0.1) was added to one half of the root system. After 12 h, one side of the split-root system was infected with 100 mg of *Fusarium* inoculum either on the same root half as the *Pseudomonas* treatments (Fus&CHA19; Fus&CHA0) or on the opposite side of the split-root microcosms (Fus/CHA0). The microcosms were covered with a 6 mm thick polycarbonate lid, sealed with sterile sealing mass (Terostat VII, Henkel, Düsseldorf, Germany), and held firmly in place with paper clamps. Fresh weights of shoot and both root halves were determined 7 d after inoculation with *Fusarium.*

Statistical analyses

The individual values of the partitioning of mobilized carbon for each side of the root system, and the ratio between those values, were analysed using repeated measurement one-way analysis of variance (ANOVA), with the treatment as categorical predictor and time as repeated factor. Data for each time point were then analysed with an independent one-way ANOVA followed by Tukey's HSD test. Statistical analyses were carried out using STATISTICA 6.0 (Statsoft, Tulsa, OK, USA).

Results

Effect of Fusarium *on C partitioning*

Inoculation with *F. graminearum* always led to successful root infection, clearly visible by brown necrotic spots on the treated roots (Fig. 1). Importantly, pathogens were restricted to the treated root half; in plants where one root half was infected, the non-treated half showed no visible fungal infection, nor were hyphae detected in its hydroponic solution.

In plants where either one or both root halves were infected, the root partitioning of ^{11}C tracer was unaffected within 28 h compared with control plants (Fig. 2a). However, infecting only one root half by *Fusarium* distorted the ^{11}C distribution between the root halves (Fig. 2b). The ^{11}C partitioning to the untreated root half (Fus–) was significantly increased by 33, 35, and 46% by 23.5, 24.5, and 28.0 h after infection, respectively, compared with root halves of control plants. At the same time, partitioning to the infected root half (Fus+) declined and was reduced by 28% at 28 h after infection, compared with control plants. Consequently, the ratio (r) between partitioning to each root half of infected compared with uninfected roots [r = (^{11}C partitioning to Fus+)/(^{11}C partitioning to Fus–)] was significantly affected, while the ratio r for control plants remained stable over time (Fig. 2b, Table 2).

Direct effects of P. fluorescens *CHA0 and CHA19 on C partitioning*

Root inoculation with either the wild-type strain CHA0 or the *gacS* mutant CHA19 only did not significantly affect ^{11}C partitioning over 30 h. Neither the ^{11}C allocation from the shoot to the entire root system, nor the partitioning between treated and untreated root halves differed significantly from control plants (data not shown).

Interactions between P. fluorescens *and* F. graminearum

In all tested combinations, *F. graminearum* successfully colonized the infected root halves in the presence of *P. fluorescens* as revealed by brown necrotic spots on the infected roots. Similarly to the single treatments with either *F. graminearum* or *Pseudomonas* (CHA0 or CHA19), carbon allocation from the shoot to the *entire* root system was unaffected by all of the double treatments with *P. fluorescens* and subsequent *F. graminearum* infection on either root half. However, pre-inoculation of either root half with *Pseudomonas* strain CHA0 strongly altered the effect of *F. graminearum* on carbon partitioning. Pre-inoculation of a root half with the wild-type strain CHA0 2 d prior to inoculation of those same roots by *F. graminearum* (Fus&CHA0) completely suppressed the reduction in carbon partitioning to the *F. graminearum*-infected roots. No differences in ^{11}C partitioning between the root portions compared with control plants were observed over 28.5 h (Fig. 3; Tables 1, 2). A crucial result was that this annihilation of the effect of *F. graminearum* on carbon partitioning also occurred systemically when one root half was pre-inoculated with CHA0 but the other root half was subsequently inoculated with *F. graminearum* (CHA0/Fus; Fig. 3). The systemic and local effects of CHA0, in suppressing the negative effect of *F. graminearum* on carbon partitioning, were indistinguishable (Tables 1, 2).

In contrast to the effect of *P. fluorescens* CHA0, pre-inoculation of roots with the *gacS* mutant *P. fluorescens* CHA19 (CHA19&Fus) did not prevent changes in ^{11}C partitioning in response to infection with *F. graminearum* (Fig. 3). However, local pre-inoculation with *P. fluorescens* CHA19 slightly reduced the effect of *F. graminearum* on carbon partitioning. The effect was reduced by 18% after 28.5 h and this difference was intermediate between the

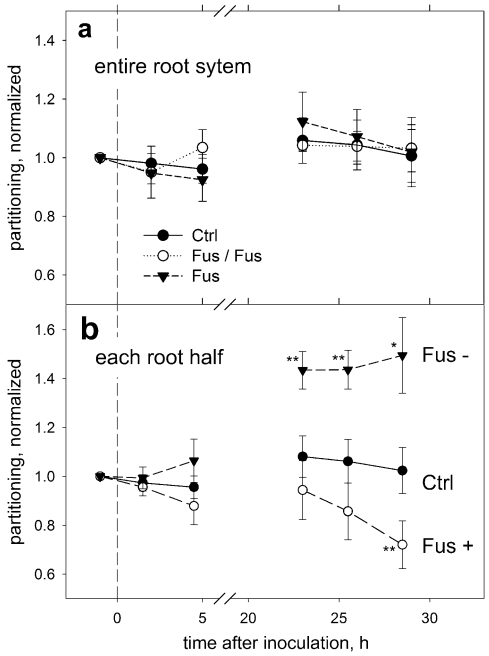

Fig. 2. Effect of *Fusarium graminearum* infection of roots in a barley split-root system on carbon partitioning from the shoot to (a) the entire root system and (b) each half of the root system. Carbon partitioning was measured as the fraction of recent photoassimilate mobilized from the $^{11}CO_2$-labelled leaf to (a) the entire root system and (b) each half of the root system. Data were normalized to the value at the time of treatment. (a) Ctrl, no infection on either root half; Fus, *F. graminearum* infection on one root half; Fus/Fus, *F. graminearum* infection on both root halves. (b) Ctrl, no infection on either root half; Fus+, *F. graminearum*-infected root half; Fus–, corresponding non-infected root half; Means ±SE, n=9 for Ctrl, n=8 for FUS, n=3 for FUS/FUS; significant differences between treatment and control were determined by Tukey's HSD test; *P <0.05; **P <0.01).

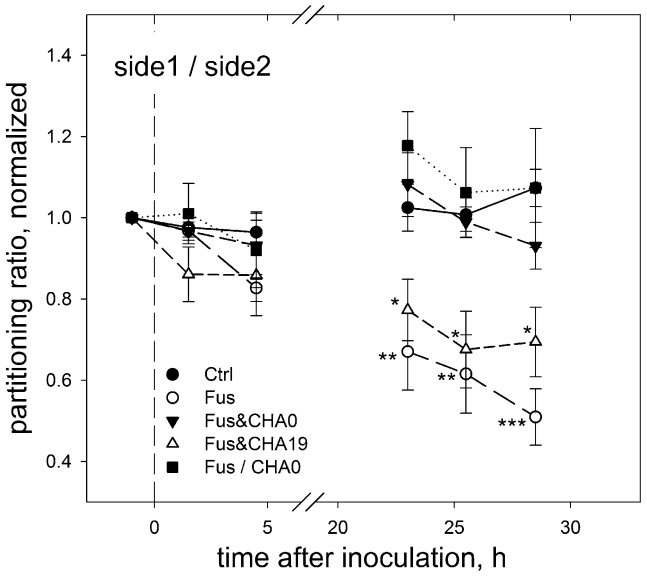

Fig. 3. Distribution of recently fixed carbon between barley root halves after infection with *Fusarium graminearum* and its modification by pre-inoculation (2 d) with the *Pseudomonas fluorescens* strains CHA0 or CHA19. Carbon partitioning was measured as the fraction of recent photoassimilate mobilized from an $^{11}CO_2$-labelled leaf to each root half. The ratio (side1/side2) is plotted, after normalization to the corresponding ratio for the plant just before treatment. Ctrl, no treatment to either root half, plotted ratio (no treatment/no treatment); Fus, treatment of one root half with *F. graminearum*, plotted ratio (*F. graminearum* treatment/no treatment); Fus&CHA0, treatment of one root half with *F. graminearum* after priming the same root half with CHA0 for 2 d, plotted ratio (*F. graminearum* plus *CHA0*/no treatment); Fus&CHA19, treatment of one root half with *F. graminearum* after priming the same root half with CHA19 for 2 d, plotted ratio (*F. graminearum* plus CHA19/no treatment); Fus/CHA0, treatment of one root half with *F. graminearum* after priming the other root half with CHA0 for 2 d, thus avoiding direct contact between micro-organisms, plotted ratio (*F. graminearum*/CHA0). Means ±SE, n=8 for Ctrl, n=8 for Fus, n=5 for Fus&CHA0, n=6 for Fus&CHA19, n=5 for Fus/CHA0. Significant differences between treatment and control were determined by Tukey's HSD test; *P <0.05; **P <0.01.).

values for the response of ^{11}C partitioning to Fus and Fus&CHA0 treatments (Fig. 3). The difference between plants with treatments CHA19&Fus and CHA0&Fus increased with time (Table 1, Fig. 3).

Effect of F. graminearum *on plant growth*

Fusarium graminearum infection together with the non-antibiotic-producing mutant *P. fluorescens* CHA19 (CHA19&Fus) on the same root half resulted in a 33% lower total plant mass after 1 week ($F_{1,16}$=12.53, P=0.002) compared with local or systemic pre-inoculation with the wild-type *P. fluorescens* CHA0 prior to *F. graminearum* infection (Table 3). Local (CHA0&Fus) or systemic (CHA0/Fus) pre-inoculation with the wild type to one part of the root system prevented the growth reduction by *F. graminearum* infection, giving indistinguishable biomasses for treated and uninfected plants (Ctrl) ($F_{1,16}$=0.98, P=0.34). Pre-inoculation with CHA0 reduced the impact of *F. graminearum* infection on the biomass of shoot and uninfected roots, but not on the infected root portion (Table 3).

Discussion

Sensitivity of the ^{11}C tracer method

The ^{11}C tracer technique proved to be ideally suited for studying the dynamic and rapid changes in partitioning of recently fixed carbon in barley plants challenged by the fungal pathogen *F. graminearum.* The method is non-invasive and therefore applicable for repeated measurements on the same plant. Further, being both quantitative and highly sensitive, it allows detection of rapid shifts in plant carbon allocation within hours, long before changes in plant biomass or morphology become apparent.

*Barley–*Fusarium *interaction*

The split-root system successfully confined microbial infections to a specific part of the root system, while the other part

Table 1. ANOVA table of F- and P-values for the effects of treatments on the relative root/root carbon partitioning over the entire experiment (repeated measures ANOVA).

Significant effects (P <0.05) are highlighted in bold. Ctrl, no treatment to either root half; Fus, *Fusarium graminearum* on one root half; Fus&CHA0, *F. graminearum* preceded by CHA0 on the same root half; Fus&CHA19, *F.graminearum* preceded by CHA19 on the same root half; Fus/CHA0, *F. graminearum* preceded by CHA0 on different root halves. Contrasts denote individual comparisons of means.

	df	*F*	*P*
Factors			
Treatment	4, 27	7.15	**<0.001**
Time	4, 27	3.15	**0.017**
Treatment×time	16, 108	3.60	**<0.001**
Contrasts			
Ctrl versus Fus	1, 27	15.49	**<0.001**
Fus&CHA0 versus Ctrl	1, 27	0.01	0.910
Fus&CHA0 versus Fus&CHA19	1, 27	6.22	**0.019**
Fus&CHA0 versus Fus/CHA0	1, 27	0.38	0.539

Table 2. Ratio between the partitioning to each root's half at the different time points.

Values for one time point that are followed by the same letter do not differ significantly (Tukey's HSD test, P <0.05). Ctrl, no treatment to either root half; Fus, *Fusarium graminearum* on one root half; Fus&CHA0, *F. graminearum* and CHA0 on the same root half; Fus&CHA19, *F. graminearum* and CHA19 on the same root half; Fus/CHA0, *F. graminearum* an CHA0 on different root halves.

	Time points				
Treatments	**1.5 h**	**4.5 h**	**23.0 h**	**25.5 h**	**28.5 h**
Ctrl	1.02 a	1.02 a	1.05 a	1.02 a	0.98 ab
Fus	0.96 a	0.81 a	0.63 b	0.56 c	0.47 c
Fus&CHA0	0.97 a	0.93 a	1.08 a	0.99 ab	0.93 ab
Fus&CHA19	0.86 a	0.86 a	0.77 ab	0.68 bc	0.69 bc
Fus/CHA0	1.01 a	0.92 a	1.11 a	1.06 a	1.07 a

remained uninfected. This permitted the detection of shifts in carbon allocation to each root half, which otherwise would have remained undetected because infection of one root portion with *F. graminearum* had no effect on total shoot/root allocation of tracer. Instead, the infection led to a rapid reduction in carbon partitioning from the shoot to infected roots, with a corresponding benefit to the uninfected roots. This shift was already visible 4.5 h after inoculation with *F. graminearum*. Using the same experimental split-root system, the application of the stress hormone jasmonic acid also resulted in a redirection of carbon to the untreated root, but it was considerably faster and already detectable within 20–30 min (Henkes *et al.*, 2008). Both results suggest that resources from the shoot are rapidly reallocated to a less stress-exposed portion of the root system as part of an active first line of defence until further defence mechanisms are effective (Lanoue *et al.*, 2010). In contrast to *F. graminearum* infection or jasmonic acid treatment, cooling of part of the root system which reduces sink capacity does not result in such compensatory allocation of carbon to the non-cooled root portion (Henkes *et al.*, 2008). The redirection of carbon to the untreated root portions in response to jasmonic acid treatment or *F. graminearum* infection therefore implies the participation of another mechanism, in addition to a reduced sink capacity of the treated roots (Henkes *et al.*, 2008). These results suggest that a fast recognition of the pathogen during infection affects the entire plant and may precede the induction of pathogen response pathways. Microarray data have demonstrated that inoculation with *F. graminearum* can change gene expression within hours: defence-related genes are up-regulated during early fungal stress (Bernardo *et al.*, 2007) and PR proteins are systemically expressed (Pritsch *et al.*, 2001), and plant-wide adjustments in carbon distribution may be part of this first line of defence. Nevertheless, it was found that *F. graminearum* led to a lower plant weight of the shoot and both parts of the root system 1 week after infection, suggesting that the pathogen overcame the plant defences and successfully established a parasitic interaction.

The allocation of photoassimilates is often altered during interactions with symbionts and pathogens. Infection with arbuscular mycorrhiza increases the sink capacity of the infected root half for carbon (Lerat *et al.*, 2003). Similarly, biotrophic pathogens distort allocation of photoassimilates to their advantage (Hancock and Huisman, 1981), but this behaviour has not been reported for necrotrophs. Allocation of shoot carbon away from infected and towards uninfected barley roots was shown. This suggests a defence strategy where the plant immediately shuts off resource supply to infected roots upon pathogen recognition, analogous to the hypersensitive response to local infections that may limit pathogen growth (Kombrink and Schmelzer, 2001). The total below-ground carbon allocation did not change, but disproportionately more energy and resources were translocated to non-infected roots.

Pseudomonas–Fusarium *interaction*

Pseudomonads can reduce plant infection not only by direct pathogen inhibition (Compant *et al.*, 2005), but also by activating plants defences (Pieterse *et al.*, 2003). Results of the presented split-root experiment showed that no direct contact between bacteria and pathogenic fungi was required to reduce infection symptoms, indicating that the effects of bacteria on plant defences may be more important than the direct toxicity of bacteria against the pathogen. Pre-inoculation of either the infected or the distant root half with *P. fluorescens* CHA0 reduced both the early symptoms of the infection (distortion of carbon allocation upon pathogen attack) and the long-term impact of the infection on plant growth. Local and systemic effects were of similar strength, suggesting that the induction of systemic resistance mechanisms was as efficient as direct contact with the pathogen. Various non-pathogenic root-colonizing microorganisms, including pseudomonads, can antagonistically affect *Fusarium* wilt. ISR of plants is important in this interaction (van Loon *et al.*, 1998). For example, a systemic response was observed in a hydroponically grown tomato culture, where prior inoculation of one root

Table 3. Effect of *Fusarium graminearum* and *Pseudomonas fluorescens* on the fresh weight of barley after 7 d.

Values for one time point that are followed by the same letter do not differ significantly (Tukey's HSD test, $P < 0.05$). Ctrl, no treatment to either root half; Fus&CHA19, *F. graminearum* and CHA19 on the same root half, the other root half remains untreated as 'control root side'; Fus&CHA0, *F. graminearum* and CHA0 on the same root half, the other root half remains untreated as 'control root side'; Fus/CHA0, *F. graminearum* and CHA0 on different root halves (Fus as 'infected root side'/CHA0 as 'control root side').

Treatments	Fresh weight of barley plant parts (mg)		
	Control root side	Infected root side	Shoot
Ctrl	65.40±11.33 a	67.20±13.55 a	175.40±10.77 a
Fus&CHA19	26.00±6.00 b	32.20±6.58 b	131.00±17.74 b
Fus&CHA0	58.00±6.53 a	31.80±4.94 b	180.40±16.90 a
Fus/CHA0	69.40±12.64 ab	33.20±7.55 b	185.60±18.22 a

compartment with a non-pathogenic *Fusarium* strain reduced the extent of infection of the other root compartment by a pathogenic strain (Fuchs *et al.*, 1997). The fluorescent pseudomonads induce systemic resistance via the jasmonic acid pathway (Iavicoli *et al.*, 2003; Pieterse *et al.*, 2003). In addition, the production of toxins such as DAPG have important side effects on plant growth (Brazelton *et al.*, 2008) and exudation of amino acids by the roots (Phillips *et al.*, 2004). In cucumber, inoculation of one side of the root system with pseudomonads impairs the growth of *Pythium aphanidermatum* on non-treated roots (Chen *et al.*, 1998), and different pseudomonads induce resistance against the pathogenic fungus *Fusarium oxysporum* in the same plant system (Liu *et al.*, 1995). The present results indicate that plants can rapidly adapt to complex and changing multipartite biotic interactions. Interestingly, it was observed that although the antibiotic-deficient strain *P. fluorescens* CHA19 did not completely suppress the effect of *F. graminearum* on carbon partitioning, it still reduced the early distortion of the carbon flow upon infection. CHA19 mutants do not produce antifungal compounds such as pyrrolnitril or HCN (Blumer *et al.* 1999), nor do they produce DAPG which is suspected to induce systemic resistance in plants (Iavicoli *et al.*, 2003). The modest suppressive effect of this strain therefore suggests that additional mechanisms may be involved in this plant–bacteria interaction. The interactions are likely to be even more complicated. Recently, it was shown that infected plant roots seem able to alert pseudomonads via systemic responses on distant root parts and stimulate their DAPG production upon infection (Jousset *et al.*, 2011). The response of plants to maintain carbon supply to infected roots in the presence of pseudomonads therefore could be interpreted as investment in defence. Since systemic responses reduce the impact of fungal infections it might be more advantageous to direct root carbon sources to pseudomonads in order to ward off fungal pathogens than to risk direct support for the fungus. From basic evolutionary principles, one could alternatively assume that the pseudomonads may show a selfish behaviour redirecting root carbon resources to themselves and risking that the fungal pathogen benefits as well.

In conclusion, this study demonstrates that barley responds rapidly to the presence of pathogens by shutting down the carbon supply to infected roots within a day. Immediate reduction of the carbon supply to attacked roots may slow down the development of the pathogen until PR genes are expressed, while non-infected roots are being supplied with proportionally higher amounts of energy and resources to ward off fungal attack. Plants that had been primed with *P. fluorescens* CHA0 did not show distortion of carbon allocation upon infection, and were less affected in their development. The antibiotic-deficient mutant strain *P. fluorescens* CHA19 only marginally protected the plant, suggesting that bacterial exoproducts are key components of the elicitation of plant defences. Further, the results show that the biocontrol effect of *P. fluorescens* does not require direct contact with the pathogen, and that systemic induction of plant defences is sufficient to protect the plant.

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