# INTERACTROOT: Molecular basis for root-root interactions between wheat and weed

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# 1 Background

# In fields, crop plants interact with individuals from their own species or weeds. The outcome of these interactions can be beneficial, neutral, or detrimental [1]. Root-root interactions are particularly interesting as they may directly interfere with the acquisition of essential soil resources (water and nutrients). Previous studies have described a number of species-specific patterns of root-root interaction [2, 3]. In some species, roots proliferate in the presence of neighbouring roots [4], while others tend to avoid root-dense patches [3]. Some aspects of root-root interactions relate to nutrient availability, although recent evidence suggests other mechanisms such as the release and detection of specific chemicals from roots may be involved [5, 6, 7]. The ability to detect roots from competing neighbours may offer a strong advantage, as root growth and maintenance are costly. While these experiments help us to understand the pattern of root growth, we don’t understand how it is achieved, especially for cereals. Root-root interactions are a topic of fundamental importance for agricultural research. Crops that efficiently compete against weeds may require fewer fertiliser and herbicide applications. Understanding the impact of competition on root growth and resource capture may lead to more sustainable systems of production for food, feed and biofuel. This project concentrated on wheat, which is a major EU cereal crop that is cultivated worldwide and whose production exceeded 682 Mt in 2009 (http://faostat.fao.org). Its productivity is threatened by the blackgrass weed (*Alopercurus myosuroides*) which is now reported to be resistant to herbicides. Our objective was to establish whether blackgrass inhibits wheat growth through root-root interactions.

## 2 Developing a robust, low-cost platform for analyzing root-root interactions

## “Hereward” was selected as a representative winter wheat that is susceptible to competition by blackgrass. We wanted a robust, and low-cost platform that enabled roots of both wheat and blackgrass to be measured in terms of main (“seminal”) root length, plus number and lengths of roots emerging from the main root (“laterals”). This is termed the “Root System Architecture” (RSA). This platform was realized by using “Magenta boxes”. These are transparent polycarbonate vessels that can be sterilized before use. We used a defined and sterilized nutrient medium solidified with a gelling agent as a proxy for soil. This allowed us to see the roots and also allowed us to manipulate the level of nutrients so we could rule out effects due to competition for this resource (Figure 1). We could remove a plug of medium and replace it with a plug of medium containing higher or lower nutrient levels before sowing the test seed on the plug. Plants were grown at controlled temperature and with a long daylength to simulate northern European latitudes. Plants were easily harvested manually. Roots were weighed and then scanned using a flatbed scanner to facilitate analysis. Root lengths were measured using ImageJ, an open access image processing tool (rsbweb.nih.gov/ij/) and RSA was quantitated using the SmartRoot computer programme [8]. This root analysis platform was used to generate the results described in subsequent sections. A second system remains under development. Here, plants are grown in a glass cylinder filled with transparent and solidified nutrient medium. To build a 3D image of the roots during growth, the cylinder is rotated on a specially designed turntable set in front of a camera. Optical problems were overcome by immersing the cylinder in a tank of water. Software is now being developed to process the images.

**3 Blackgrass alters the root system architecture of wheat**

Wheat was grown as a single plant, in competition with other wheat plants or in competition against blackgrass or *Brachypodium distachyon* (a “model” grass for studies). We found no evidence for shoot interactions influencing root interactions. Competition did not affect seminal root length but significantly changed the numbers and lengths of the wheat’s lateral roots (Figure 1). When wheat competed against wheat, numbers of lateral roots were unaffected but their lengths were shortened. This suggests that a wheat plant can recognize the presence of another wheat plant and then they stop their roots from growing into one another. However, both grasses stopped wheat from producing normal numbers of lateral roots and inhibited lateral outgrowth. The degree of inhibition depends on the type of grass. Changing nutrient supply did not affect the root-root interactions, suggesting that chemicals secreted by the weed roots (“exudates”) were having a direct effect on wheat roots. The overall effect of this inhibition of wheat root growth by grass exudates will be a restriction of water and nutrient capture, leading to a reduction in crop yield. Changes in RSA could be driven by changes in cell calcium as a messenger, signalling the perception of exudates. We collected exudate from blackgrass roots and tested them on the roots of another model plant (*Arabidopsis thaliana*) that had been modified genetically to produce the jellyfish protein aequorin [9]. This protein emits light when it binds to cell calcium and so can be used as a measure of changes in calcium. When we challenged Arabidopsis roots with blackgrass exudates, we observed increases in cell calcium, consistent with a signal’s being generated. We now need to produce wheat plants with aequorin to test for causal links between blackgrass exudates, changes in calcium and RSA modification. Identification of the specific chemicals in blackgrass exudates that change calcium and inhibit wheat root growth will be an important next step in developing strategies to maintain crop production. This will potentially benefit the EU’s wheat breeders, farmers and ultimately consumers.

**4 Career progression**

Dr. Swarbreck has successfully re-integrated into European research and established international collaborations. She has gained further funding for her work from the Newton Trust and the Gatsby Charitable Foundation. Dr. Swarbreck has supervised students from the UK and France. Her work on root interaction is now being prepared for publication. Dr. Swarbreck has been awarded a three year personal Research Fellowship by the Broodbank Trust to continue her research on root interactions at the University of Cambridge (http://www.plantsci.cam.ac.uk/directory/swarbreck-stephanie).

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