ROTHAMSTED RESEARCH

*Q&A for Bruce et al. study: The first crop plant genetically engineered to release an insect pheromone for defence – Thursday 25th June 14:00 BST*

## Q1: What was the aim of the GM wheat trial at Rothamsted?

The aim was to test the hypothesis that a GM wheat, modified to express an aphid alarm pheromone called (*E*)-β-farnesene (EβF), might act to repel aphids in the field. Aphids are a serious pest of arable crops in the UK, carrying viral diseases and reducing yields, and farmers are forced to spray broad-spectrum insecticide to control them. The idea was to work with nature, using the natural alarm pheromone produced by aphids when under attack by predators. If this were to have a repellent effect, wheat might be able to 'protect' itself and farmers might be able to reduce the use of insecticides, benefiting the environment, the farmer and making arable farming overall more sustainable.

## Q2: What are the results of the field experiment?

GM wheat plants were successfully modified to produce the aphid alarm pheromone in significant quantities without major unexpected changes seen in the appearance or performance of the new wheat plants, which looked and yielded as normal. The GM wheat clearly demonstrated a repellent effect in the laboratory, but in the open field trial there was no statistically significant difference observed between the GM wheat expressing EβF and the control wheat in terms of numbers of aphids observed.

## Q3: Has the trial been a failure?

Every experiment is designed to test a hypothesis. In order to do credible tests that will give a reliable answer one needs to have controls and needs to test the experimental “subject” against the controls. If the experiment is well designed, and all the controls are in place, then one can test a hypothesis. This is normal scientific practice. The experiment itself was successful, because it allowed us to test the hypothesis of whether the GM wheat could repel aphids in the field. It provided a conclusive answer that the wheat did not repel aphids, but that is not the same as a failed experiment or failed science.

The experiment has certainly raised additional questions rather than provided simple answers! The discrepancy between laboratory biology and the agronomy of the field is a frequent occurrence in plant science and so is not completely unexpected. Conditions in the field are much more complicated and variable than in the laboratory. For example, the growing conditions during 2012 and 2013 when the experiments were taking place were not ideal - the number of aphids was low due to wet and cold conditions during the summer. This would have reduced the likelihood of obtaining a statistically significant result if the wheat were to be having any repellent effect. We are considering future work to discover more about this - see Q5.

## Q4: Does this mean that GM in crops does not work?

Actually the genetic engineering component of the project was entirely successful - the wheat plants were engineered with DNA sequences that allowed them to express EβF as intended, and without any observed negative effect on other aspects of crop performance. This in itself is an important discovery and proof of concept, opening up the possibility of using pest defences in plants to displace the use of chemical insecticides in agriculture.

In addition, it is important to understand that whatever the results of this project, all GM crops need to be assessed on a case by case basis. There are many different traits - from insect resistance to herbicide tolerance to drought tolerance to nitrogen efficiency - already available in crops to farmers around the world, and which are working extremely well. Genetic modification is simply a technical process for very precise plant breeding, and its results should be judged on the merits of each case.

## Q5: What will happen now?

Given that this project has raised more questions than answers, there are some clear lines of potential future enquiry. We have learnt that in order to repel natural aphid populations in the field, we may need to alter the timing of release of the alarm signal from the plant to mimic more closely the release by the aphid, which happens in short bursts in response to a threat. This may require altering release rates of alarm pheromone from the plants, but also engineering the wheat plant to release the pheromone only when the aphid arrives. We might also consider conducting trials in more diverse environments, particularly where high populations of aphids and their natural enemies can be expected. This has been a valuable study in terms of the knowledge that we acquired and we are looking into developing a follow up project proposal to refine the approach.

## Q6: Is the GM wheat safe to eat?

We did not evaluate our GM wheat for use as food and feed as this was beyond the scope of this study. This was a first-round proof-of-concept experiment and even if the initial results had shown a major repellent impact on aphids in the field, we would still have had to do a lot of work to proceed towards full commercialisation.

## Q7: How much money was spent on the project?

The project was funded entirely by the UK Government through the Biotechnology and Biological Sciences Research Council (BBSRC) to an initial value of £1,176,000. The research cost of the whole project was £732,000.00, in line with the normal costs of a 5-year research project of this nature. £444,000 was invested in fencing to use in this and future research trials. The fencing protects the site from intruders as well as preventing wild animals from entering the trial site.

Unfortunately, because of repeated threats by protestors to destroy the trial, and the intrusion to the trial site by an individual who caused criminal damage with an attempt to destroy the trial in 2012, we had to increase security, which resulted in a significant increase in costs. An additional £1,794,439 was funded by the BBSRC for security measures at Rothamsted Research to protect this and other research trials. It is sad that such a high expenditure is still necessary to protect scientific experiments against damage and vandalism by those who oppose science, innovation and progress.

## Q8: What are the technical details of the project?

All the technical details, involving experimental design, methodology, genetics, agronomic performance and associated data are freely available to anyone. They have been published in the open-access paper Bruce et al, 2015, 'The first crop plant genetically engineered to release an insect pheromone for defence', in the journal *Scientific Reports*. To access it follow this link (after Thursday 25th June 14:00h) http://nature.com/articles/doi:10.1038/srep11183. While the majority of papers published in scientific journals are behind paywalls that bar anyone without a subscription from accessing the paper, we wanted to ensure that the details of this experiment were openly available. To further facilitate accessibility of the technical details see also Q8.1-Q8.5 below.

### Q8.1 How did you make the GM plants?

We took small samples of wheat plants and physically inserted the genes into the cells. The genes to be inserted were coated onto the surface of very small particles of gold shot into wheat cells using a device that is sometimes called a "gene gun". We then used the cells containing the new genes to make seedlings in the lab. We actually made lot of different transgenic plants using both the 'gene gun' and another method involving a soil bacterium *Agrobacterium tumefaciens* but the lines possessing the highest (*E*)-β-farnesene emission were plants made using the gene gun and these were chosen for the field trial.

### Q8.2 Where did the genes come from?

The genes we inserted into the wheat plants were produced by chemical synthesis and not taken from another plant or animal. The gene that makes (*E*)-β-farnesene, encodes a protein that is similar to that found in peppermint but versions of this gene are also present in many other plants. The other enzyme required (farnesyl pyrophosphate synthase) had similarity to that found in cow (*Bos taurus*) and was encoded by a synthetic and optimised gene sequence. We chose a non-plant form of the farnesyl pyrophosphate synthase gene to minimise the chances of the gene or its product being made inactive from the host plant. Very similar approaches (using FPPS from birds) have previously shown to work well in transgenic plants engineered to make related volatile chemicals.

**Q8.3 Could you see any differences between normal wheat plants and the GM ones?**

GM plants and control wheat plants look identical. They can be distinguished by techniques that compare the DNA they contain or by analysing the (*E*)-β-farnesene they give off.

**Q8.4 The GM plants give off another compound (myrcene) at higher levels than the non-GM plants that are hosts to aphids. What does this mean?**

As we generated a new GM Wheat plant we wanted to thoroughly examine its biochemistry. For this reason, we rigorously analysed the GM plants. The production of myrcene was not surprising as it has a very similar chemical structure to EBF and is made by the same processes in the cell. Myrcene is often found in aphid host plants but it has no role as a host plant cue in this instance. When we breed plants conventionally we also see changes in their biochemistry.

**Q8.5 The study showed that the aphids become less responsive to the chemical when exposed to it for some time. What does this mean?**

Habituation to prolonged exposure would not occur due to it being released by a GM plant, but could eventually occur however the EBf was delivered. The plants used in this study were producing the alarm signal constantly, and one improvement would be to change them to produce the chemical only when they are attacked, or only at the more vulnerable growth stages. This more limited exposure would reduce the chance of the pests being constantly stimulated by and eventually ignoring the alarm signal.

The trait developed in this study had to be tested in the field against natural pest and predator populations as the lab experiments under controlled environment conditions are indicators only. The results provide us with knowledge to build upon. This is normal rigorous scientific practice.

Pest control measures in agriculture need to evolve and change in relation to continued efficacy and breakdown of resistance mechanisms, this is true for pesticide formulations as well as resistant plant varieties regardless of whether they came from GM modification or conventional breeding.

## Q9: Why has it taken so long to publish the results?

We finished the fieldwork in December 2013, after which we had a large amount of experimental data to process and analyse. We then wrote up the results in a draft manuscript, as agreed by all the co-authors, for peer review and publication. This process commonly takes many months – that is the norm with scientific publications. The early release of data before full publication is not considered good practice in the scientific community.