PART B: INFORMATION ABOUT THE RELEASE APPLICATION TO BE INCLUDED ON THE PUBLIC REGISTER

B1 The name and address of the applicant

Rothamsted Research,
West Common, Harpenden
Hertfordshire,
AL5 2JQ
UK

B2 A general description of the genetically modified organisms in relation to which the application is being made

The organisms to be released are wheat plants that have been genetically modified to test a novel resistance to aphids, a major pest of cereals. The genetically modified plants were made by inserting new DNA into the wheat genome using a micro-particle delivery system.

The new genes added encode enzymes that lead to the production of a volatile chemical that is naturally produced by aphids and many other plants. This chemical, (*E*)-β–farnesene (EBF) is known to repel aphids and to attract their natural enemies such as parasitoids and predators to the plant. The two new genes are synthetic i.e. they were not taken from another organism but chemically synthesized to function like wheat genes. The proteins they encode are common in nature and the particular forms used here are similar to those found in peppermint and cow.

The plants also contain two selectable marker genes which both originate in bacteria. The *bar* gene gives the plant resistance to glufosinate herbicides and was used in the selection of transgenic plants. The *nptI* gene confers resistance to the antibiotic kanamycin and was used in the gene cloning steps. Glufosinate will not be used to control weeds on the trial site and this antibiotic resistance gene is not considered harmful in the context of this trial.

B3 The location at which the genetically modified organisms are proposed to be released

The location of the field trial is an agricultural area forming part of an experimental farm at Rothamsted Research Harpenden, UK and at grid reference TL 1213. The area for the proposed field trial, including controls and spacing between GM plots will cover 80x80m however the area planted with GM wheat is only 288 square meters.

B4 The purpose for which the genetically modified organisms are proposed to be released.

Current methods to control aphids in agricultural crops rely on chemical insecticides. We have genetically engineered wheat plants to give off a volatile chemical (EBF) which is used by aphids under attack as an alarm pheromone or signal and causes other aphids to stop feeding and move away from the source. In addition, emission of EBF would be expected to cause increased foraging by predators and aphid parasitoids. We have studied these genetically modified plants in the laboratory and have already demonstrated that they repel aphids and attract the natural parasitoids and predators. The purpose of this trial is to test whether these plants are better able to resist aphids under field conditions.

The aims of the trial are:

1) To evaluate the effect of the non-toxic aphid repellent (*E*)-β–farnesene as a approach to

crop protection that does not rely on agricultural inputs (pesticides).
2) To confirm that (*E*)-β–farnesene that is produced by these GM wheat plants still functions in a 'real life' situation (i.e. in a field as opposed to a lab/greenhouse).
3) To determine the effect on colonization of the wheat crop by natural populations of three species of cereal aphid under real field conditions. We expect populations to be suppressed.
4) To evaluate effects on aphid specific predators and parasitoids (e.g. aphid parasitoids, ladybirds, hoverflies, lacewing larvae). These effects are expected to be positive unlike broad spectrum insecticides which do collateral damage to natural enemy populations
5) To determine effects on any other pest species present.
6) To investigate the persistence of plant DNA in the soil

B5 The intended dates of the release.

The exact timing of sowing of the trial will depend upon weather conditions at the time. The field trial start date will be in March or April 2012 and the plants will be harvested in Aug or Sept the same year. To increase the statistical robustness of the data we intend to repeat the trial with the same GM lines in 2013.

B6 The environmental risk assessment.

The two GM lines are indistinguishable from their non-GM equivalents except for the volatile emission of (*E*)-ß-farnesene. Where applicable, the gene donor organisms are not known to be pathogenic or allergenic and neither the genes under investigation, nor the selectable marker genes are expected to result in the synthesis of products that are harmful to humans, other organisms or the environment. Any unknown hazards arising from the expression and ingestion of foreign proteins will not be realised because the wheat plants will not be consumed by humans.

The probability of seeds escaping from the trial site or the transfer of inserted characteristics to sexually-compatible species outside the trial area is estimated as very low. Commercial wheat varieties do not establish easily or thrive in uncultivated environments and are naturally self-pollinating with out-crossing being a rare event. Wheat seeds are relatively large and not normally dispersed by wind. Management measures including netting when the wheat is in ear and the use of gas guns and hawk kites will be employed to mitigate the risk of seed removal by birds. Management procedures to minimise the spread of seeds or pollen will further reduce the probability of these events occurring. There will be no cereals grown for 80 meters from the boundary of the site and no sexually-compatible wild relatives of wheat exist in the vicinity. If out-crossing to plants outside the trial area where to somehow occur, selection pressure to maintain the genes in the environment would exist only where glufosinate-based herbicides were applied. Even if the emission of (E)-ß-farnesene provides excellent protection from aphids at the field level (the trait under evaluation in these trials), the chances of successful establishment of these wheat plants in unmanaged ecosystems is extremely low and this would still be the case under severe infestations of aphids.
The risk of non-sexual, horizontal gene transfer to other species is extremely low. In the event of horizontal gene transfer to bacteria, neither the trait genes nor the selectable marker genes would be expected to confer a selective advantage in the field environment under consideration. The plasmid backbone sequences, nptI gene, origins of replication, border sequences etc. come originally from E coli and Agrobacterium tumefaciens, two common gut and soil bacteria respectively and these sequences are already widespread in the soil metagenome. Although this makes potential homologous recombination events more likely, we estimate the likelihood of horizontal gene transfer as low and the consequences, were it to occur, as negligible. The area proposed to be planted with GMOs is small; eight 6x6m plots and temporary (lasting between 5 and 6 months).
Although the above-ground plant material will be cleared from the site, the npt1 gene contained in the plant root DNA will decompose into the soil. The transgene is fully integrated

into the plant DNA and the copy number is low thus the npt1 gene represents a very small proportion (much less than one millionth) of the total DNA in any one cell of our transformed wheat plants. This excess of competing DNA will significantly dilute the rate of any npt1 natural bacterial transformation. In addition, enzymatic degradation of free plant DNA in the soil and the low level of spontaneous bacterial competence to take up free DNA will significantly reduce the incidence of natural transformation. Although the transfer of functional gene units from plants to soil bacteria is accepted to be extremely low under natural conditions, it cannot be completely discounted that some bacteria may successfully take up the npt1 gene. However, there will be no antibiotics applied to the soil to provide additional selection pressure for the gene to persist in the environment. The source of the nptI gene is the gut bacterium E. coli carrying a plasmid containing the transposable element (Tn 903). R plasmids possessing resistance to aminoglycoside antibiotics are already naturally found in the soil and other environments. The npt1 gene encodes the enzyme Aminoglycoside 3'-phosphotransferase which confers resistance to kanamycin and related aminoglycoside antibiotics. Although these antibiotics still have some clinical applications, alternatives are readily available. Taken together, and bearing in mind the limited scope of this trial, the risk of generating of any additional antibiotic resistance within the soil microbial community or risks to human health or the environment if this were to occur as a result of the proposed trial is considered to be extremely low.
The overall risk of harm to human health or the environmental arising from this trial is assessed as very low.

B7 The methods and plans for monitoring the genetically modified organisms and for responding to an emergency.

The release site will be visited by trained laboratory personnel who are working on the project at no less than weekly intervals (and at some periods, daily) during the growing season of each year of the trial. Any unexpected occurrences that could potentially result in adverse environmental effects or the possibility of adverse effects on human health will be notified to the Defra immediately. Should the need arise to terminate the release at any point the emergency plans detailed below will be followed.

At the end of each season, the plot will remain in stubble and monitored for volunteers during the remainder of the year and the following season. Any volunteers identified will be destroyed by herbicide treatment (e.g. glyphosate) or removed by hand and destroyed.

Following completion of the two-year trial the release site will remain fallow for a further season to enable easy identification of volunteers. The site will be inspected regularly and any volunteers identified will be immediately destroyed either by application of a systematic broad leaf herbicide.

Emergency procedures: In the unlikely event that the integrity of the site is seriously compromised, the trial will be terminated and all plants, (including GM and control wheat plots, pollen barrier rows and barley separators) will be destroyed using a suitable herbicide or harvesting as deemed appropriate. All harvested material will be removed from the site and disposed of by incineration or deep burial at a local authority-approved landfill site using an approved contractor. Transportation of waste materials will be in secure containers. The phone numbers of all key staff will be available to site security and farm.