APPLICATION FOR CONSENT TO RELEASE A GMO - HIGHER PLANTS

PART A1: INFORMATION REQUIRED UNDER SCHEDULE 1 OF THE GENETICALY MODIFIED ORGANISMS (DELIBERATE RELEASE) REGULATIONS 2002

PART 1

General information

1. The name and address of the applicant and the name, qualifications and experience of the scientist and of every other person who will be responsible for planning and carrying out the release of the organisms and for the supervision, monitoring and safety of the release.

Applicant:

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

2. The title of the project.

Study of aphid, predator and parasitoid behaviour in wheat producing aphid alarm pheromone

PART II

Information relating to the parental or recipient plant

3. The full name of the plant -

(a) family name, Poaceae

(b) genus, Triticum

(c) species, aestivum

(d) subspecies, N/A

(e) cultivar/breeding line, Cadenza

(f) common name. Common wheat/ bread wheat

- 4. Information concerning -
 - (a) the reproduction of the plant:
 - (i) the mode or modes of reproduction,
 - (ii) any specific factors affecting reproduction,
 - (iii) generation time; and
 - (b) the sexual compatibility of the plant with other cultivated or wild plant species, including the distribution in Europe of the compatible species.
- ai) Reproduction is sexual leading to formation of seeds. Wheat is approximately 99% autogamous under natural field conditions; with self-fertilization normally occurring before flowers open. Wheat pollen grains are relatively heavy and any that are released from the flower remain viable for between a few minutes and a few hours. Warm, dry, windy conditions may increase cross-pollination rates on a variety to variety basis (see also 6 below).
- aii) Pollination, seed set and grain filling are dependent on temperature, weather conditions, agronomic practice and pressure applied by pests and disease.
- aiii) The generation time is 20-25 weeks. For Cadenza sown as a spring-wheat type, one season is normally from March/April to August /September. For Cadenza sown as a winter wheat, one season is normally Aug/Oct to August/September
- b) Wheat is naturally self-pollinating but under experimental conditions wheat can be crossed with various wild grasses. Of these, only the genera *Elymus* and *Elytrigia* (formerly *Agropyron*) are present in the UK but there are no reports of wheat x *Agropyron* spontaneous hybrids. Wheat can also be forced using laboratory techniques to cross to rye, triticale and a limited number of other cereals.
- 5. Information concerning the survivability of the plant:
 - (a) its ability to form structures for survival or dormancy,
 - (b) any specific factors affecting survivability.
- 5 a) & b) Wheat is an annual species and survives from year to year only via seed production. In normal farming practice, mature seeds may fall from the plant prior to or at the time of harvest and not be collected. If not managed, these seeds may over-winter in the soil and germinate the following spring as 'volunteers'. Cadenza is a UK milling variety, which is photoperiod-sensitive (ppd-D1) but has a negligible vernalising requirement and relatively high levels of frost tolerance which means it can be sown either as a spring or winter type with good frost-tolerance under typical UK winter conditions (Whaley et al 2004).
- 6. Information concerning the dissemination of the plant:
 - (a) the means and extent (such as an estimation of how viable pollen and/or seeds decline with distance where applicable) of dissemination; and
 - (b) any specific factors affecting dissemination.

Pollen can be disseminated by the wind. Such dissemination is limited by the relatively large size and weight of wheat pollen. The risk of cross-pollination is also reduced by its short period of viability. Reports quantifying the rate of cross pollination state that out-crossing rates are usually less that 1% (eg. Hucl 1996). Under certain growing conditions individual genotypes may have out-crossing rates of up to 4-5% (Griffin 1987; Martin 1990). Seed is usually retained by the plant until harvest but a small proportion can be spilt to the ground at that time. Dispersal of seed prior to harvest by wind is unlikely, but possible by wildlife.

7. The geographical distribution of the plant.

Wheat is grown in temperate zones worldwide, mainly in Europe, North America and Asia.

8. Where the application relates to a plant species which is not normally grown in the United Kingdom, a description of the natural habitat of the plant, including information on natural predators, parasites, competitors and symbionts.

N/A

9. Any other potential interactions, relevant to the genetically modified organism, of the plant with organisms in the ecosystem where it is usually grown, or elsewhere, including information on toxic effects on humans, animals and other organisms.

Wheat plants have a range of pests and fungal pathogens. The main insect pests in the UK are three aphid (Homoptera: Aphididae) species, the bird cherry-oat aphid, *Rhopalosiphum padi*, the grain aphid, *Sitobion avenae*, and the rose grain aphid, *Metopolophium dirhodum*, the orange wheat blossom midge, *Sitodiplosis mosellana* (Diptera: Cecidomyiidae)and wheat bulb fly *Delia coarctata* (Diptera: Anthomyiidae). Wheat also interacts with beneficial insects for example *Aphidius rhopalosiphi* (Hymenoptera: Aphidiinae) which attack aphid pests.

Wheat is not toxic and a major world bulk commodity food but may cause gastro-intestinal intolerance or 'bakers' asthma' in susceptible individuals.

Plants and seeds arising from this trial will not enter the food and feed chains.

PART III

Information relating to the genetic modification

10. A description of the methods used for the genetic modification.

Transgenic wheat plants were produced using standard protocols by microprojectile bombardment Sparks & Jones (2009).

The gene(s) of interest were maintained on separate plasmid vectors each containing a bar gene selectable marker cassette and bombarded on gold particles into scutella of immature zygotic embryos. Whole plants were regenerated and selected from somatic embryos induced in tissue culture.

11. The nature and source of the vector used.

The genes of interest were carried on a binary vector pBract309 (www.bract.org). This was prepared in *E. coli* Invitrogen DH5 α sub-cloning-efficiency competent cells (Genotype F- ϕ 80lacZ Δ M15 Δ (lacZYA-argF)U169 recA1 endA1 hsdR17(rk-, mk+) phoAsupE44 thi-1 gyrA96 relA1 λ -)

Plasmids were purified using a Qiagen plasmid purification Midi kit resulting in the following concentrations: pBract309EBFS+T (clone 6) 1.5mg/ml and pBract309FPPS+T (clone 1) 0.7mg/ml.

12. The size, intended function and name of the donor organism or organisms of each constituent fragment of the region intended for insertion.

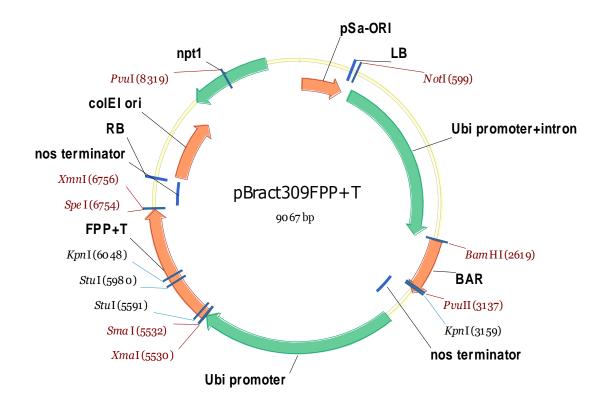
Chemically synthesised gene sequences that had been codon-optimised for wheat encoding plastid-targeted enzymes (E)- β -farnesene synthase and farnesyl diphosphate synthase were assembled by GenScript Inc. NJ, USA and introduced into plant cells on complete binary plasmids by biolistic transformation (see Tables and note below). Genes encoding (E)- β -farnesene synthase and farnesyl diphosphate synthase both possessed a wheat chloroplast transit sequence from the small subunit of RubisCo, previously validated to correctly target the proteins to wheat plastids (Primavesi *et al* 2008). The nucleotide sequences of these genes are synthetic and chimaeric and not found naturally. However, the enzyme encoded by the EBFS cassette is similar to that found in peppermint ($Mentha \times piperita$) and the enzyme encoded by the FPPS cassette has most similarity to that from cow ($Bos\ taurus$) but is generally ubiquitous and occurs in most organisms. Both plasmids carry right and left T-DNA border sequences, origins of replication and bacterial selectable marker genes necessary for maintenance in E.coli and Agrobacterium.

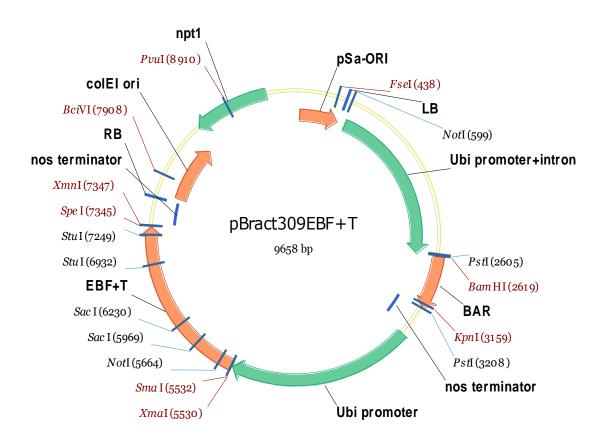
pBract309EBFS+T

Element	Size	Donor Organism	Description and Intended Function	
ColE1	723bp	E.coli	Origin of replication for plasmid replication in <i>E. coli</i>	
pSa-Ori	483bp	Agrobacterium	Origin of replication for plasmid replication in	
		tumefaciens	Agrobacterium	
nptI	800bp	E.coli	Bacterial selection gene conferring resistance to	
(aph(3')-la)			Kanamycin and other antibiotics	
RB	24bp	Agrobacterium	T-DNA Right border	
		tumefaciens		
LB	23bp	Agrobacterium	T-DNA Left border	
		tumefaciens		
<i>Ubi</i> +intron	2kb	Zea mays	Maize ubiquitin 1 promoter + first intron driving	
			constitutive expression in wheat	
EBFS	1.665kb	Synthetic	A synthetic gene encoding E-β farnesene synthase,	
			codon optimised for wheat	
Chloroplast	147bp	Wheat (Triticum	Pre-sequence from the small subunit of RubisCo to	
targeting		aestivum)	provide protein targeting to plastids (inc	
sequence			chloroplasts)	
nos T	300bp	Agrobacterium	Nopaline synthase terminator for gene of interest	
		tumefaciens		
Bar coding	480bp	Streptomyces	Plant selectable marker gene encoding	
sequence		hygroscopicus	phosphinothricin acetyltransferase conferring	
			resistance to herbicides with active ingredient	
			glufosinate ammonium. (N.B. <i>Bar</i> gene	
			inefficient/inoperative in this plasmid)	

pBract309FPPS+T

Element	Size	Donor Organism	Description and Intended Function	
ColE1	723bp	E.coli	Origin of replication for plasmid replication in E. coli	
pSa-Ori	483bp	Agrobacterium	Origin of replication for plasmid replication in	
		tumefaciens	Agrobacterium	
nptl	800bp	E.coli	Bacterial selection gene conferring resistance to	
(aph(3')-Ia)			kanamycin and other antibiotics	
RB	24bp	Agrobacterium	T-DNA Right border	
		tumefaciens		
LB	23bp	Agrobacterium	T-DNA Left border	
		tumefaciens		
<i>Ubi</i> +intron	2kb	Zea mays	Maize ubiquitin 1 promoter + first intron driving	
			constitutive expression in wheat	
FPPS	1.074kb	Synthetic	A synthetic gene encoding farnesyl pyrophosphate	
			synthase, codon optimised for wheat	
Chloroplast	147bp	Wheat (Triticum	Pre-sequence from the small subunit of RubisCo to	
targeting		aestivum)	provide protein targeting to plastids inc chloroplasts	
sequence				
nos T	300bp	Agrobacterium	Nopaline synthase terminator for gene of interest	
		tumefaciens		
<i>Bar</i> coding	480bp	Streptomyces	Plant selectable marker gene encoding	
sequence		hygroscopicus	phosphinothricin acetyltransferase conferring	
			resistance to herbicides with active ingredient	
			glufosinate ammonium. (N.B. Bar gene	
			inefficient/inoperative in this plasmid)	





PART IV

Information relating to the genetically modified plant

13. A description of the trait or traits and characteristics of the genetically modified plant which have been introduced or modified.

The volatile sesquiterpene (E)- β -farnesene (EBF) 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. It also acts as a repellent to colonising aphid morphs. In addition, emission of EBF would be expected to cause increased foraging by predators and aphid parasitoids (Beale *et al.* 2006).

EBF synthase converts substrates including farnesyl diphosphate (FPP) into EBF. We expressed EBF synthase in transgenic wheat plants and, to increase the substrate pool available, we generated additional lines that also expressed FPP synthase. Both the EBF synthase and the FPP synthase were driven by a constitutive promoter and targeted to the chloroplast.

The plasmids used also contain the Ubi1::bar::nos cassette which confers resistance to herbicides with active ingredient glufosinate ammonium but this was used only to select transgenic plants and this trait will not be utilised in proposed field trials.

- 14. The following information on the sequences actually inserted or deleted:
 - (a) the size and structure of the insert and methods used for its characterisation, including information on any parts of the vector introduced into the genetically modified plant or any carrier or foreign DNA remaining in the genetically modified plant,
 - (b) the size and function of the deleted region or regions,
 - (c) the copy number of the insert, and
 - (d) the location or locations of the insert or inserts in the plant cells (whether it is integrated in the chromosome, chloroplasts, mitochondria, or maintained in a non-integrated form) and the methods for its determination.

We propose to include two GM events in the field trial. Event 2803R6P1 contains one copy of the pBract309EBFS+T plasmid and one copy the pBract309FPPS+T plasmid per haploid genome, as determined by quantitative (Taqman) PCR performed on genomic DNA by iDNA Genetics (Norwich, UK) using regions of the coding sequence as primers/probe. Event 2812R9P1 contains 4 copies per haploid genome of plasmid pBract309EBFS+T only. Segregation analysis using PCR to genomic DNA indicates that in both lines, the insertions are stably inherited in the chromosomal DNA and that the lines selected for field trial are homozygous.

We have not analysed the position or the structure of the insertion nor sequenced the flanking genomic DNA. Apart from the expected phenotype of EBF emission, these plants are indistinguishable from untransformed controls. No other changes to the plant morphology or development are apparent.

- 15. The following information on the expression of the insert -
 - (a) information on the developmental expression of the insert during the lifecycle of the plant and methods used for its characterisation,
 - (b) the parts of the plant where the insert is expressed, such as roots, stem or pollen.

The EBF synthase (EBFS), FPP synthase (FPPS) and bar genes are under the transcriptional control of the maize Ubi1 promoter which is known to give constitutive expression in wheat. We have not measured the levels or activity of the newly expressed enzymes but we know that these plants emit EBF at levels of between 30ng/plant/h and 1833ng/plant/h.

16. Information on how the genetically modified plant differs from the parental or recipient plant in the following respects -

- (a) mode or modes and/or the rate of reproduction,
- (b) dissemination,
- (c) survivability.

Except for the emission of EBF, all aspects of the phenotype of events 2803R6P1 and 2812R9P1 including morphology, pollination and seed-set appear to be identical to non-transgenic control wheat plants. We would expect dissemination of pollen and seeds to be the same as for non-transgenic wheat plants. The survivability of these plants in unmanaged systems may be affected by their ability to modify the behaviour of aphids and their parasitoids or predators. In addition, these plants possess the ability to tolerate glufosinate-based herbicides which would increase their survivability in environments where these herbicides were the only ones used.

17. The genetic stability of the insert and phenotypic stability of the genetically modified plant.

We have not specifically investigated genetic or phenotypic stability of these lines but all plants expressing the transgene are morphologically indistinguishable from untransformed controls. The inheritance of the transgene over three generations follows normal rules of Mendelian genetics.

18. Any change to the ability of the genetically modified plant to transfer genetic material to other organisms.

It is expected that the events 2803R6P1 and 2812R9P1 would not differ from conventional wheat in their capacity to self or cross pollinate via sexual reproduction (see parts 4 and 6). A low rate (approximately 1%) of cross pollination with closely adjacent wheat plants within the trial is anticipated. The individual plots are separated from each other by 10 m (0.5m space, 9m barley, 0.5m space) and from the edge of the trial by 10 meters of barley (or space) plus a 3m pollen barrier of wheat.

Event 2803R6P1 contains DNA from the plasmids pBract309EBFS+T and pBract309FPPS+T, and event 2812R9P1 has integrated DNA from the plasmid pBract309EBFS+T only. Both these plasmids possess a bacterial origin of replication and antibiotic resistance and we have assumed that these are present in the plant genomic DNA. These elements may increase the rates of horizontal gene transfer and establishment in soil bacteria because they provide a theoretical mechanism for homologous recombination and selection (if aminoglycoside antibiotics are present). However, we estimate the rate of horizontal gene transfer is low and, if it were to occur, these genetic elements are already present in bacteria and in soil microbes in particular.

19. Information on any toxic, allergenic or other harmful effects on human health arising from the genetic modification.

EBF occurs naturally in plants as well as being produced by aphids as a warning signal: trace amounts are even produced by wild type wheat (Buttery et al, 1985). We have extensively used this compound in semiochemical field trials albeit from artificial dispensers and not by biosynthetic production by the crop itself. EBF is highly volatile and unstable compound and therefore would not accumulate in the environment. The transgenic wheat lines we developed emit EBF in gas phase at rates of approximately 1833 ng/plant/h for event 2803R6P1 and 30 ng/plant/h for event 2812R9P1. There appears to be no published toxicity or allergenicity data for EBF but at the levels expected to be generated by these plants and because they will not enter the food or feed chains, we consider the potential toxic or harmful effects to be negligible. EBF occurs in many food and fragrances. For example, it is a component of beers as a consequence of its occurrence in the essential oil of hops. In total, over 400 plant species, including edible examples (potato, grapevine), are known to produce EBF (see appendix I and http://www.pherobase.net/database/floral-compounds/floral-taxa-compounds-detail-E-beta-farnesene.php).

Furthermore, EBF breaks down rapidly in air particularly with sunlight. It is decomposed to benign oxidation products. The first products of oxidative decomposition are EBF epoxides and diols. The next layer of decomposition, mostly oxidative again, gives acetone, at very low levels, and benign low molecular weight organic mono- and di- carboxylic acids. Then further decomposition takes place to a number of even more ubiquitous even lower molecular weight compounds.

In plants, FPP is part of the terpenoid biosynthetic pathway and is one of the substrates for the synthesis of sesquiterpenes including (E)-ß-farnesene. It is a widely occurring plant and animal metabolite. Indeed, FPP plays a role in human metabolism (Wilkin *et al* 1990). It is an intermediate in the pathway for cholesterol synthesis, serves also as precursor for synthesis of various non-steroidal isoprenoids. There appears to be no published toxicity or allergenicity data for FPP but because this compound is ubiquitous in many organisms and the plants will not enter the food or feed chains, we consider the potential toxic or harmful effects to be negligible at the levels expected to be found in this trial.

20. Information on the safety of the genetically modified plant to animal health, particularly regarding any toxic, allergenic or other harmful effects arising from the genetic modification, where the genetically modified plant is intended to be used in animal feeding stuffs.

None of the plant material from the field trial will enter the human food- or animal feed-chain.

21. The mechanism of interaction between the genetically modified plant and target organisms, if applicable.

The target organisms in this case are both aphid pests of wheat which we predict will be repelled by the odours of the transformed plants and the predators and parasitoids of aphids which we predict will be attracted to the EBF plots. The alarm pheromone is a warning signal that aphids release when attacked by predators to alert other neighbouring aphids. Aphids rapidly move away when they detect EBF. In addition, emission of EBF would be expected to increase the foraging behaviour of predators and aphid parasitoids. We will produce EBF in the trial crop which we anticipate will repel aphids and make them more vulnerable to predation. EBF has a non-toxic mode of action and has very specific effects on aphid behaviour. Furthermore, EBF serves as an attractant for beneficial insects which attack aphids which further enhances the crop protection effect.

22. The potential changes in the interactions of the genetically modified plant with non-target organisms resulting from the genetic modification.

We are not aware of any non-target effects. EBF is an alarm pheromone which is specific to aphids and acts as a kairomone for aphid parasitoids and predators.

23. The potential interactions with the abiotic environment.

None.

24. A description of detection and identification techniques for the genetically modified plant.

PCR using primers specific for EBFS and/or FPPS genes.

25. Information about previous releases of the genetically modified plant, if applicable.

None.

PART V

Information relating to the site of release

(Applications for consent to release only)

26. The location and size of the release site or sites.

The area for each proposed field trial, including controls and spacing between GM plots will cover 80x80m. We propose to carry out two spring-sown trials and one autumn-sown trial over a two year period on two adjacent sites within a fenced area covering approximately 3 ha. It will be sited in the farm at Rothamsted Research, Harpenden, UK and at grid reference TL 120130. Each trial sowing will include 8 6x6m plots (288 m2) planted with events 2803R6P1 or 2812R9P1 plus 8 6x6m plots of non-transgenic controls. Each plot will be separated from each other by 10m (0.5m space, 9m barley, 0.5m space) and from the edge of the trial by 13m separator strip cultivated with barley plus walkways or wheat to include a 3m wheat pollen barrier which will completely surround the trial (see Figure in Section 34). Enclosing the whole site will be a 2.4m high chain-link fence (with lockable double gates) and within that a second fence also with lockable gates to prevent the entry of rabbits and other large mammals including unauthorised humans.

27. A description of the release site ecosystem, including climate, flora and fauna.

The release site is an agricultural area forming part of an experimental farm. The flora and fauna are typical of agricultural land in the South East.

28. Details of any sexually compatible wild relatives or cultivated plant species present at the release sites.

Wheat is a self-pollinating crop with very low rates of cross-pollination with other wheat plants. The only wild relatives of wheat commonly found in the UK are in the genera *Elymus* and *Elytrigia* (formerly *Agropyron*) although there are no reports of cross-hybridisation between wheat and these genera. The two most common inland species are *Elytrigia repens* (common couch = *Agropyron repens*) and *Elymus caninus* (bearded couch = *Agropyron caninum*). Other related species, such as *Elytrigia juncea* (Sand couch = *Agropyron junceum*), *Elytrigia atherica* (Sea couch = *Agropyron pycnanthum*) and hybrids are largely confined to coastal habitats.

E. repens is common on the Rothamsted estate whereas *E. caninus* is less common and is confined to woods and hedgerows. *E. repens* propagates primarily by vegetative reproduction (rhizomes), rather than by sexual reproduction, and in any case, no reports of wheat x *Elytrigia* or *Elymus* spontaneous hybrids have been reported. *E. repens* will be controlled along with other

weeds in and around the trial site using standard farm practices. No wheat or other cereals will be grown within 80m from the trial.

29. The proximity of the release sites to officially recognised biotopes or protected areas which may be affected.

There are no protected areas near the trial site.

PART VI

Information relating to the release

30. The purpose of the release of the genetically modified plant, including its initial use and any intention to use it as or in a product in the future.

This is a research trial to assess any change in behaviour of aphids, their parasitiods or predators that result from the modified volatiles given off by these GM plants.

31. The foreseen date or dates and duration of the release.

In 2012 the plants will be sown in March/April and harvested in July/Aug/Sept.

In 2013 the plants will be sown in March/April and harvested in July/Aug/Sept. Plants will also be sown in Sept/Oct 2013 and terminated in Nov 2013 / Early 2014

32. The method by which the genetically modified plants will be released.

Seeds will be drilled using conventional plot-scale farm equipment.

33. The method for preparing and managing the release site, prior to, during and after the release, including cultivation practices and harvesting methods.

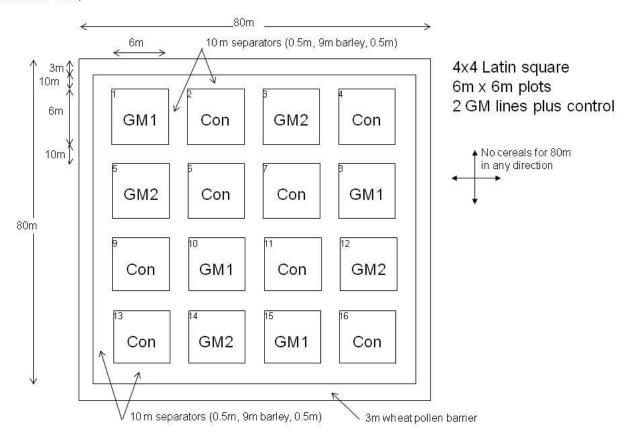
The site will be prepared according to standard agronomic practices for spring or winter wheat cultivation as appropriate. For the two spring-grown trials, the release will be monitored regularly during all stages of development and will be harvested just prior to full maturity (target growth stage of cereal decimal code 87). The 2013 winter-sown trial will also be monitored regularly and the immature plants will be killed by spraying with a broad-spectrum herbicide. Some seeds from the spring-grown GM and control plots will be conditioned, threshed and stored in appropriate GM seed stores. All other seeds from the spring-sown trials, including that from the pollen barrier rows and barley will be disposed of to deep landfill using an approved contractor. All straw will be chopped and left on site.

34. The approximate number of genetically modified plants (or plants per square metre) to be released.

See trial design. GM plants will be sown in eight plots of 6X6m. the other plots and guard rows will be sown with non-GM wheat of the same variety as the GM plots. The separators will be non-GM wheat or barley. Planting density will be appropriate to achieve approximately 300 plants per m²

Proposed GM wheat field trial design

(not draw to scale)



PART VII

Information on control, monitoring, post-release and waste treatment plans

35. A description of any precautions to

(a) maintain the genetically modified plant at a distance from sexually compatible plant species, both wild relatives and crops.

Wheat is a self-pollinating crop with very low rates of cross-pollination with other wheat plants. The only wild relatives of wheat commonly found in the UK are in the genera *Elymus* and *Elytrigia* (formerly *Agropyron*). The two most common inland species are *Elytrigia* repens (common couch = *Agropyron repens*)and *Elymus caninus* (bearded couch = *Agropyron caninum*). Other related species, such as *Elytrigia juncea* (Sand couch = *Agropyron junceum*), *Elytrigia atherica* (Sea couch = *Agropyron pycnanthum*) and hybrids are largely confined to coastal habitats.

E. repens is common on the Rothamsted estate whereas *E. caninus* is less common and is confined to woods and hedgerows. *E. repens* propagates primarily by vegetative reproduction (rhizomes), rather than by sexual reproduction, and in any case, no reports of wheat x *Elytrigia* or Elymus spontaneous hybrids have been reported. *E. repens* will be controlled along with other weeds in and around the trial site using standard farm practices. The outer edge of the trial has a 13m barrier of non-GM wheat and barley which will minimise pollen spread pollen from the GM plots. No wheat or other cereals will be grown within 80m from the trial.

(b) any measures to minimise or prevent dispersal of any reproductive organ of the genetically modified plant (such as pollen, seeds, tuber).

The outer edge of the trial has a 13m barrier of non-GM wheat and barley which will minimise pollen spread from the GM plots. The drills will be filled on the trial area and will be thoroughly cleaned before leaving the trial area. Glufosinate-based herbicide will not be used in the trial. To minimise the possibility of seed loss, the spring-grown plants will be harvested just prior to full maturity (with a target growth stage of decimal code 87). A sample of plants will be hand-harvested, conditioned and threshed to supply seeds for the following year's trial or research purposes. The remaining grain obtained will be disposed of to deep landfill using an approved contractor. All straw will be chopped and left on site.

36. A description of the methods for post-release treatment of the site or sites.

The trial will receive standard farm practise as regard to herbicide, fungicides and nitrogen in conjunction with the scientific co-ordinator. The site will be regularly monitored from sowing to harvest and during the following cropping year.

37. A description of the post-release treatment methods for the genetically modified plant material including wastes.

For harvesting the spring-sown trials, a sample of the plots will be collected with a plot combine to obtain yield measurements. The grain sampled will be analysed on site at Rothamsted Research, all samples taken from the field will be closely monitored and records kept of weights and movements of grain and straw. All small samples removed from the trial site will eventually be destroyed by an approved technique. The remainder of the site will be harvested by either a commercial combine or the plot combine. The grain obtained will be disposed of to deep landfill using an approved contractor. All straw will be chopped and left on site. The combine will be cleaned in the empty half of the fenced area prior to leaving the site so that all traces of gm plant material will remain in the trial area. The trial area will remain in stubble for the following year to enable monitoring of volunteers and a broad spectrum herbicide such as glyphosate will be applied as required. The autumn-sown trial will not be harvested but terminated by spraying with herbicide.

38. A description of monitoring plans and techniques.

The three trials will be monitored regularly (at least weekly) during the respective growing periods and after the termination of the trials during the following year. Records will be kept of each visit.

39. A description of any emergency plans.

In the unlikely event that the integrity of the site is seriously compromised, the trial will be terminated and all plants destroyed using a suitable herbicide or burning on site as deemed appropriate. The phone numbers of all key staff will be available to site security and farm.

40. Methods and procedures to protect the site.

A Hertfordshire Constabulary have been notified that we intend to carry out GM field trials at Rothamsted Research in the near future and we have started discussions with them as regard to site security. We propose to fence a total area of approximately 160m x 80m with a 2.4m high chain-link fence (with lockable double gates) and to have movement-activated cameras and a manned security presence.

PART VIII

Information on methodology

- 41. A description of the methods used or a reference to standardised or internationally recognised methods used to compile the information required by this Schedule, and the name of the body or bodies responsible for carrying out the studies.
- 1. DNA synthesis was provided by GenScript Inc. USA http://www.genscript.com/index.html
- 2. Standard molecular biology reagents and methods were used following Sambrook et al., (1989).
- 3. Wheat transformation was performed using biolistics as described in Sparks and Jones, (2009)
- 4. Transgene copy number and zygosity testing was provided via Taqman PCR by iDNA Genetics Norwich UK. http://www.idnagenetics.com/
- 5. Volatile production by transformed plants was determined using air entrainment (sampling of the headspace around the plant) and Gas Chromatography techniques as described in Beale et al., (2006)
- 6. Behavioural responses of target aphid species to volatiles from transformed plants were determined using bioassay techniques described in Beale et al., (2006)

References

Beale MH, Birkett MA, Bruce TJA, Chamberlain K, Field LM, Huttly AK, Martin JL, Parker R, Phillips AL, Pickett JA, Prosser IM, Shewry PR, Smart LE, Wadhams LJ, Woodcock CM and Zhang YH (2006) Aphid alarm pheromone produced by transgenic plants affects aphid and parasitoid behavior. Proc Natl Acad Sci U S A 103:10509-10513.

Bruce TJA, Birkett MA, Blande J, Hooper AM, Martin JL, Khambay B, Prosser I, Smart LE & Wadhams LJ (2005) Response of economically important aphids to components of Hemizygia petiolata essential oil. Pest Management Science 61: 1115-1121.

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PART A2: DATA OR RESULTS FROM ANY PREVIOUS RELEASES OF THE GMO

Events containing these genes have not previously been released.

PART A3: DETAILS OF PREVIOUS APPLICATIONS FOR RELEASE

Rothamsted Research has received two previous consents to release GM wheat:

97/R8/3 and 01/R8/4

PART A4: RISK ASSESSMENT AND A STATEMENT ON RISK EVALUATION

Summary

Observations on the general plant morphology of glasshouse-grown plants, timing of flowering, fertility, seed shape and germination show that the two GM wheat events 2803R6P1 and 2812R9P1 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 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*)-*B*-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, *nptl* 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).

The overall risk of harm to human health or the environmental arising from this trial is assessed as *very low*.

Detailed evaluation of hazards, magnitude of exposure and management strategies to minimise risk.

We adopted a classic six-step process of risk assessment. Systematic identification of all potential hazards arising from this field trial; evaluation of hazard-realisation in the specific field-trial environment; potential for harm; frequency of exposure; mitigation of risk by appropriate management and finally, an estimate of the overall risk.

Step1: Potential hazards which may be caused by the characteristics of the novel plant	Step 2: Evaluation of how above hazards could be realised in the receiving environments	Step 3: Evaluation the magnitude of harm caused by each hazard if realised	Step 4: Estimation of how likely/often each hazard will be realised as harm	Step 5: Modification of management strategies to obtain lowest possible risks from the deliberate release	Step 6: Overall estimate of risk caused by the release
Increased invasiveness in natural habitats or persistence in agricultural habitats due to inserted trait.	Increased invasiveness may arise from intended or unintended effects of the genetic modification that resulted in wheat plants with a more 'weedy' habit that are better able to establish and thrive in uncultivated environments or to persist in agricultural habitats.	Wheat is an annual species that requires active management to out-compete more weedy plants. Left unmanaged, wheat does not establish and survive in nature and thus has a low base line of invasiveness and persistence. Even if intended or unintended effects of the genetic modification resulted in major changes in invasiveness or persistence, it is considered that this would not result in significant environmental harm for agricultural or unmanaged ecosystems. Wheat is a benign plant that can be easily managed by cultivation or herbicides. The magnitude of harm if the hazard was realised is considered to be very small.	It is highly unlikely that intended or unintended effects of the genetic modification will result in major changes in invasiveness or persistence. If it were to occur, this hazard would be realised only if seeds or pollen possessing genes encoding these traits were to spread from the trial site and successfully become established elsewhere. This is very unlikely as wheat pollen is relatively heavy so does not travel far, it has a short half-life and there are no sexually compatible species for out-crossing for at least 80m from the trial site. Seed removal from the site will be rigorously managed (see step 5). The chances of modified wheat plants establishing themselves outside the trial site are negligible.	Harvested seeds will be transported from the site in sealed containers. Machinery will be cleaned thoroughly prior to removal from the site. There is a large buffer zone to minimize the spread of pollen. Surrounding the trial site is an 80 metre area in which no cereals will be grown so it will be easy to see any cereal plants in the surrounding area. Appropriate physical barriers and/or deterrents will be employed to minimise access by large mammals and birds. Glufosinate herbicides will not be used on the trial site.	Overall risk is negligible.
Selective advantage or disadvantage conferred to wheat or other sexually compatible plant species.	Selective advantage or disadvantage may result from the intended traits (improved resistance to aphids and tolerance to	The basal ability for commercial cereal crop varieties to survive in uncultivated environments is very low. We anticipate that the conferred trait of improved resistance to aphids will provide only minor selective advantage compared to other factors determining a plants ability to survive in unmanaged ecosystems. The genetic modification resulting in increased	This hazard would be realised only if seeds or pollen possessing genes encoding these traits were to spread from the trial site and successfully become established in environments were the appropriate selection pressures were present. This is very unlikely as wheat pollen is	Harvested seeds will be transported from the site in sealed containers. Machinery will be cleaned thoroughly prior to removal from the site. There is a large buffer zone to minimize the spread of pollen. Surrounding the trial	Overall risk is very low.

Potential environmental impact due to interactions between the novel plant and non-target organisms	glufosinate herbicides) or as a result of unintended effects of the genetic modification. These hazards could be realised in the receiving environment via dispersal of GM seeds from trial site to the surrounding environment or via out-crossing to sexually-compatible species outside trial site. EBF may illicit a change in behaviour of non- target organisms.	tolerance to glufosinate herbicides has the potential to confer a major selective advantage only where those herbicides are used routinely. EBF is highly specific to aphids and their natural enemies so the magnitude of harm resulting from interactions between nontarget organism and the modified plants is negligible.	relatively heavy so does not travel long distances, it has a short half-life and there are no sexually compatible species for outcrossing for at least 80m from the trial site. Seed removal from the site will be rigorously managed. Heavy infestations of aphids that would provide a significant selective advantage are rare, however the use of glufosinate herbicides in the surrounding agricultural fields may be expected. Overall, the frequency of this hazard resulting in environmental harm is very low. The frequency of contact between non-target organisms and the modified plants or the EBF given off by them is expected to be high. However, as EBF is not likely to be in the repertoire of recognisable compounds of nontarget organisms, contact is not expected to cause a change in behaviour.	site is an 80 metre area in which no cereals will be grown so it will be easy to see any cereal plants in the surrounding area. Appropriate physical barriers and/or deterrents will be employed to minimise access by large mammals and birds. Glufosinate herbicides will not be used on the trial site. In the very unlikely event of major disturbances of insect behaviour become apparent, the trial will be terminated.	Overall risk is very low.
Potential effect on human or animal health due to introduced <i>E</i> - beta-farnesene (EBF)	By contact or ingestion of GM plant material.	Although there are no robust toxicity data available for EBF, it is considered that the magnitude of harm caused by contact, inhalation or ingestion of these GM plants is extremely low. EBF occurs in many food and fragrances. For example, it is a component of beers as a consequence of its occurrence in the essential oil of hops. EBF is known to occur naturally in a range of plants as well as being produced by aphids as a warning signal: trace amounts are even produced by wild type wheat	Some contact between the GM plants and humans or animals is expected. People operating farm machinery and scientists working in the trial site will come into physical contact with the plants. Small mammals such as mice, invertebrates and birds may also come into contact and/or ingest plant material. However, the volatility of EBF means that it will not accumulate in the seeds or	No plant material from the trial will enter the food or animal feed chain. Appropriate physical barriers and/or deterrents will be employed to minimise access by large mammals and birds. Machinery will be cleaned before being removed from	Overall risk is very low.

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		(Buttery et al, 1985). In total, over 400 plant species, including several edible types, are known to produce EBF (http://www.pherobase.net/database/floral-compounds/floral-taxa-compounds-detail-E-beta-farnesene.php). In the quantities produced by the GM plants, EBF is not considered harmful. Furthermore, EBF breaks down rapidly in air particularly with sunlight. It is decomposed to benign oxidation products. The first products of oxidative decomposition are EBF epoxides and diols. The next layer of decomposition, mostly oxidative again, gives acetone, at very low levels, and benign low molecular weight organic mono- and di- carboxylic acids. Then further decomposition takes place to a number of even more ubiquitous even lower molecular weight compounds.	other plant material.	the trial site	
Potential effect on human or animal health due to introduced farnesyl diphosphate (FPP)	By contact or ingestion of GM plant material.	Although there are no robust toxicity data for FPP available, it is considered that the magnitude of harm caused by contact, inhalation or ingestion of these GM plants is extremely low. In plants, FPP is part of the terpenoid biosynthetic pathway and is one of the substrates for the synthesis of sesquiterpenes including (E)-ß-farnesene. It is a widely occurring plant, animal and prokaryote metabolite. It occurs widely in nature. FPP plays a role in human metabolism (Wilkin et al (1990), where it is an intermediate in the pathway for cholesterol synthesis and serves as precursor for synthesis of various nonsteroidal isoprenoids. It is not considered toxic or harmful at the levels found in these events.	Some contact between the GM plants and humans or animals is expected. People operating farm machinery and scientists working in the trial site will come into physical contact with the plants. Small mammals, invertebrates and birds may also come into contact and/or ingest plant material.	No plant material from the trial will enter the food or animal feed chain. Appropriate physical barriers and/or deterrents will be employed to minimise access by large mammals and birds. Machinery will be cleaned before being removed from the trial site	Overall risk is very low.

Potential effect on human or animal health due to introduced phosphinothricin acetyl transferase (PAT)	By contact or ingestion of GM plant material.	The magnitude of harm caused by contact, inhalation or ingestion PAT in these GM plants is extremely low. The source organism for this gene (Streptomyces hygroscopicus) is ubiquitous in the soil and there have been no reports of its adverse affects on humans, animals or plants. The product of the bar gene, phosphinothricin acetyl transferase (PAT) has been evaluated on numerous occasions by EFSA and found to raise no safety concerns. For example: "The enzyme phosphinothricin acetyl transferase (PAT) is not likely to present safety problems. Its enzymatic function is specific to a substrate which is not naturally present in humans, namely phosphinothricin, and furthermore, it is degraded and inactivated in simulated gastric fluid containing pepsin at pH 1-1.2. It is therefore unlikely to retain any enzymatic activity in vivo. Furthermore, no sequence homology between the PAT protein and known toxins has been found. The native PAT protein (51% purity) has been tested for acute toxicity in mice and no toxicity has been reported at a dose of 5 g per kg body weight."	Some contact between the GM plants and humans or animals is expected. People operating farm machinery and scientists working in the trial site will come into physical contact with the plants. Small mammals, invertebrates and birds may also come into contact and/or ingest plant material.	No plant material from the trial will enter the food or animal feed chain. Appropriate physical barriers and/or deterrents will be employed to minimise access by large mammals and birds. Machinery will be cleaned before being removed from the trial site	Overall risk is very low.
Potential direct effect on human or animal health due to introduced neomycin phosphotransf erase	By contact, inhalation or ingestion of GM plant material.	The magnitude of harm caused by contact, inhalation or ingestion of plant material containing NPTI is extremely low. The source organism for gene encoding this enzyme (<i>E. coli</i>) is present in the large intestine of healthy humans and any NPTI ingested is expected to be broken down by digestive enzymes in the stomach and small intestine. Although specific toxicity data on neomycin phosphotransferase I (also known as	The frequency of exposure is very low. The promoter driving expression of the <i>nptl</i> gene is prokaryote-specific so NPTI protein will not be present in the modified plants.	No plant material from the trial will enter the food or animal feed chain.	Overall risk is very low.

Potential	By contact,	aminoglycoside 3'-phosphotransferase type 1) could not be found, there are several studies reported in scientific literature of the safety of a functionally related enzyme NPTII. For example, acute oral toxicity of NPTII was studied in mice that had received an oral dose of 100, 1000, or 5000 mg NPTII/kg bodyweight and subsequently monitored for adverse effects over the following seven days. The authors concluded that no treatment-related adverse health effects had occurred (Fuchs et al., 1993).	The rate of horizontal gene	No plant material from the	Overall risk
effects on human or animal health due to horizontal gene transfer of recombinant DNA	ingestion or infection with bacteria that had received recombinant DNA via horizontal gene transfer.	contact, ingestion or infection with bacteria that had received the recombinant DNA via horizontal gene transfer is low. The EBF and FPP genes are not expected to be expressed in bacteria and would have no safety concern if they were. Horizontal gene transfer of a complete <i>nptl</i> fragment could confer functional antibiotic resistance to receiving bacteria. Some aminoglycoside antibiotics including kanamycin are important for clinical treatment, especially for second line treatment for multi-resistant tuberculosis (kanamycin) and in gut irrigation in, for example, encephalopathy (neomycin). However, this resistance is already widespread in the environment. The source of the <i>nptl</i> gene is the gut bacterium <i>E. coli</i> carrying a plasmid containing the transposable element (Tn 903). R plasmids possessing resistance to aminoglycoside antibiotics are already widespread in the soil.	transfer from genetically modified plants to other species is accepted to be extremely low (EFSA, 2009). However, the presence of plasmid backbone sequence and origins of replication which are derived from E. coli and Agrobacterium tumefaciens, increase the chances of homologous recombination between plant and microbial DNA in the soil. If recombinant DNA were to move by horizontal transfer to soil bacteria, it is unlikely to significantly increase the prevalence of resistance to aminoglycoside antibiotics in the environment. The area proposed to be planted with GMOs is small; a total eight 6x6m plots (288 m2) and temporary (lasting between 5 and 6 months).	trial will enter the food or animal feed chain. No antibiotics will be applied to the soil to give selective advantage.	is very low.
Potential effects on	Changes in biogeochemical	The magnitude of harm is estimated to be extremely low. Biogeochemical processes	The frequency of changes to biogeochemical processes is	None.	It is very unlikely that

biogeochemic al processes (changes in soil decomposition of organic material	processes may result from unintended changes in the modified plants or from unintended changes in soil microbes due to horizontal transfer of DNA.	are not expected to be affected by the cultivation of the genetically modified plants.	considered to be very low. The area proposed to be planted with GMOs is small eight 6x6m plots and temporary (lasting between 5 and 6 months).		changes in biogeoche mical processes would occur.
Possible environmental impact due to changes in cultivation practice	This modification may result in the application of fewer insecticidal sprays.	The magnitude of any changes due to changes in cultivation practice will be negligible. A reduction in the spraying of insecticides to control aphids is likely to have a positive effect on beneficial insects.	The frequency that this hazard may be realised is low. The number of EBF-emitting plants is small (eight 6x6m plots) and will be sown for only one growing season (between 5 and 6 months).	None.	Overall risk negligible.

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PART A5: ASSESSMENT OF COMMERCIAL OR CONFIDENTIALITY OF INFORMATION CONTAINED IN THIS APPLICATION.

Identify clearly any information that is considered to be commercially confidential. A clear justification for keeping information confidential must be given.

This is publically funded research and has no associated commercial confidentiality considerations.

PART A6: STATEMENT ON WHETHER DETAILED INFORMATION ON THE DESCRIPTION OF THE GMO AND THE PURPOSE OF RELEASE HAS BEEN PUBLISHED

Make a clear statement on whether a detailed description of the GMO and the purpose of the release have been published, and the bibliographic reference for any information so published. This is intended to assist with the protection of the applicant's intellectual property rights, which may be affected by the prior publication of certain detailed information, e.g. by its inclusion on the public register.

A description of the GMO and the purpose of the release have not yet been published.

Appendix 1: Plants reported in the scientific literature as naturally emitting (E)-ß-farnesene

Alismatales, Araceae

Sauromatum guttatum

Hadacek, F., and Weber, M. 2002. Plant Biol. 4:367-383.

Apiales, Apiaceae

Aegopodium podagraria Anthriscus sylvestris Carum carvi Heracleum sibiricum Laserpitium latifolium Meum athamanticum

Borg-Karlson, A.-K., Valterová, I., and Nilsson, L.A. 1994. Phytochem. 35:111-119.

Borg-Karlson, A.-K., Valterová, I., and Nilsson, L.A. 1994. Phytochem. 35:111-119.

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Cornu, A., Carnat, A.-P., Martin, B., Coulon, J.-B., Lamaison, J.-L., Berdague, J.-L.

2001. J. Agric. Food Chem. 49:203-209.

Borg-Karlson, A.-K., Valterová, I., and Nilsson, L.A. 1994. Phytochem. 35:111-119.

Arecales, Arecaceae

Pastinaca sativa

Aiphanes minima Asterogyne martiana Bactris gasipaes Calyptrogyne costatifrons Calyptrogyne ghiesbreghtiana Ceroxylon alpinum Chamaedorea linearis Chelyocarpus ulei Geonoma brongniartii Geonoma congesta Geonoma cuneata var. cuneata Geonoma cuneata var. procumbens Geonoma cuneata var. sodiroi Geonoma irena Geonoma longepedunculata Geonoma macrostachys var. macrostachys Geonoma maxima Geonoma orbignyana Geonoma poeppigiana Geonoma polyandra

piscicauda Geonoma stricta var. stricta Geonoma tenuissima Geonoma triglochin

Geonoma undata Iriartea deltoidea Mauritia flexuosa

Geonoma stricta var.

Pholidostachys synanthera Prestoea schultzeana Welfia regia

Wettinia kalbreyeri

Knudsen, J.T., Tollsten, L. and Ervik, F. 2001. Plant Biol. 3:642-653.

Knudsen, J.T. 1999. Mem. New York Bot. Gard. 83:141-168.

Knudsen, J.T., Tollsten, L. and Ervik, F. 2001. Plant Biol. 3:642-653.

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Knudsen, J.T. 1999. Mem. New York Bot. Gard. 83:141-168.

Knudsen, J.T., Tollsten, L. and Ervik, F. 2001. Plant Biol. 3:642-653.

Wettinia maynensis

Knudsen, J.T., Tollsten, L. and Ervik, F. 2001. Plant Biol. 3:642-653.

Asparagales, Hyacinthaceae

Hyacinthus orientalis

Brunke, E.-J., Hammerschmidt, F.-J., and Schmaus, G. 1994. Flav. Fragr. J. 9:59-69.

Asparagales, Orchidaceae

Acacallis cyanea Acacallis superba Acineta superba Aerangis appendiculata Aerangis biloba Aerangis brachycarpa Aerangis confusa Aerangis distincta Aerangis fastuosa Aerangis kirkii Aerangis kotschyana Aerangis modesta

Aerangis somalensis Aeranthes grandiflora Aerides crassifolia Aerides fieldingii Aerides jackianum

Aerides lawrenceae Aganisia pulchella **Ancistrochilus** rothschildianus Angraecopsis amaniensis Angraecum aporoides Angraecum bosseri Angraecum eburneum Angraecum eichlerianum Angraecum girymae Angraecum sesquipedale Anguloa clowesii Anguloa uniflora Anguloa virginalis Ansellia gigantea Bifrenaria flagillaris

Bifrenaria fuerstembergiana Bifrenaria thyrianthina Bifrenaria wittigii Bollea coelestis Brassavola digbyana Brassavola glauca Brassavola nodosa Brassavola tuberculata Brassia lobbii Brassia verucosa Catasetum expansum

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Kaiser, R. 1995. Pp. 135-168 in K. H. C. Baser (ed.), Proc. 13th Int. Congr. Flavours,

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Gongora galeata

Gongora grossa Gongora odoratissima Gongora pseudoatropurpurea Gongora quinquenervis Gongora saccata Gongora scaphephorus Gongora truncata Gymnadenia conopea Himantoglossum hircinum Horichia dressleri Houlletia clarae Houlletia odoratissima Houlletia tigrina Houlletia wallisii Huntleya heteroclita Huntleya lucida Huntleva meleagris Kefersteinia auriculata Kefersteinia pellita Kegeliella houtteana Lacaena bicolor Laelia albida Laelia anceps Laelia autumnalis Laelia gouldiana Laelia perinii Liparis viridiflora Listera ovata Lueddemannia pescatorei Lvcaste aromatica Lycaste cruenta Lycaste deppei Lycaste locusta Lycomormium fiskei Masdevallia caesia Masdevallia estradae Masdevallia glandulosa Masdevallia laucheana Masdevallia lehmannii Masdevallia striatella Masdevallia tridens Maxillaria nigrescens Maxillaria picta Maxillaria tenuifolia Maxillaria variabilis Miltonia regnellii Miltonia schroederiana Miltonia spectabilis Miltoniopsis phalaenopsis Miltoniopsis warscewiczii Mormodes andreetae Neofinetia falcata

Nigritella nigra

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Notylia barkeri Notylia buchtienii Odontoglossum cirrhosum Odontoglossum constrictum Odontoglossum pendulum Odontoglossum pulchellum Oncidium longipes Oncidium ornithorhynchum Oncidium sarcodes Oncidium tigrinum Ophrys bombyliflora Ophrys insectifera Ophrys scolopax ssp. attica Ophrys scolopax ssp. cornuta Ophrys speculum Ophrys tenthredinifera Orchis mascula Paphinia grandiflora Peristeria cerina Peristeria elata Peristeria guttata Pescatorea cerina Pescatorea davana Pescatorea klabochorum Pescatorea lehmannii Pescatorea wallisii Phalaenopsis violacea Platanthera bifolia Platanthera chlorantha Plectrelminthus caudatus Polycycnis gratiosa Polystachya campyloglossa Polystachya cultriformis Polystachya fallax Polystachya mazumbaiensis Rangaeris amaniensis Rhynchostylis coelestis Rodriguezia refracta Sievekingia fimbriata Sievekingia herrenhusiana Sievekingia rhonhofiae Sievekingia suavis Stanhopea annulata Stanhopea embreei Stanhopea grandiflora Stanhopea impressa Stanhopea jenischiana Stanhopea oculata Stanhopea tigrina Stenia palorae Trevoria lehmannii

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Asterales, Asteraceae

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Santolina

chamaecyparissus

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Caryophyllales, Cactaceae

Discocactus cristallophilus Discocactus silicicola Dolichothele longimamma Echinopsis coronata Echinopsis mamillosa Espostoa blossfeldiorum Harrisia adscendens Hylocereus polyrhizus Hylocereus venezuelensis Pereskia aculeata Pilosocereus arrabidae Pilosocereus catingicola Pilosocereus pachycladus Rebutia marsoneri Rebutia narvaecense Selenicereus grandiflorus Selenicereus hamatus Sulcorebutia kruegeri

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Caryophyllales, Nyctaginaceae

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