Managing bumblebee imports to maintain pollinator diversity and increase efficiency in horticultural production

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Please tick below the appropriate area on the research continuum where you feel this project fits

BASIC/FUNDMENTAL \hspace{5cm} APPLIED/PRE COMMERCIAL

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Key words: Pollination, Strawberry, Bumblebee, Risk
1. Rationale for Undertaking the Research

Bees provide the essential ecosystem service of pollination and are by far the most important insect pollinators, pollinating over 90% of the world’s 254,000 flowering plant species. Although bees are typically associated with honey production, their role as pollinators is far more important. For example, sales of Irish honey in 2008 were valued at €992,000; whereas bee pollination generated €14.4 million of horticultural produce in the same year. Furthermore, the above only reflects the direct benefits of pollination as an ecosystem service; the indirect benefits include the maintenance of sexual reproduction in a host of wild flowers and trees which provide a myriad of other services such as habitat for other beneficial insects, nutrient cycling and soil stability. Overall, current estimates of the monetary value of both direct and indirect benefits of pollination to the Irish economy range from €52-200 million p.a.

Since the mid-1990s, scientists have become increasingly concerned about declines in pollinator populations worldwide and the issue has received formal recognition within the Convention on Biological Diversity, signed by 150 governments, in the establishment of an International Pollinators Initiative (IPI) in 2000. Data from regional studies conducted as part of the IPI have confirmed that the abundance and diversity of wild bees, including wild honeybees, are now in decline and some species are close to extinction. These declines are primarily driven by fragmentation and loss of natural habitats which provide both floral and nesting resources. In Europe, both Britain and the Netherlands have lost 52% and 67%, respectively, of their bee biodiversity since 1980; and 25% of bee species in France and Belgium are in decline. In Ireland, 30% of our 101 bee species are considered threatened and three species have already gone extinct.

As the above illustrates, reliance on a single bee species for agricultural pollination is unsustainable. Fortunately, 20 years ago the horticultural industry responded by domesticating an alternative group of bees, the bumblebees. The global trade in bumblebee hives is now worth over €55 million p.a., with over one million bumblebee hives exported worldwide each year. In Europe, the domesticated species of bumblebee is *Bombus terrestris*, a species that naturally occurs throughout Europe and North Africa. Specialised facilities, primarily in the Netherlands, commercially rear the bumblebees for year-round export. However, *B. terrestris* includes a recognised number of subspecies, in particular *B. terrestris audax* which is found only in Ireland and Britain. Currently, the breeding-stock of commercial bumblebees is not native to Ireland and unregulated importation could pose a serious risk to our native species of bees.
Studies in both Canada and Japan have shown that imported bumblebees have been responsible for spreading both diseases and parasites to native bumblebees. Imported bumblebees can also escape from glasshouses and successfully compete with native bumblebees, eventually establishing themselves in areas with frequent importation. Finally, imported bumblebees may successfully breed with native *B. terrestris audax* and over time it may lose any unique physiological adaptations to Irish conditions through genetic dilution.

In recognition of both the continuing need of Irish growers to import bumblebees for pollination and the risks involved in importing non-native bees, the research objectives of the RSF funded project were:

- To improve management of imported bumblebees to increase pollination efficiency;
- To reduce drift between hives and escape from areas of importation;
- To determine the level of genetic differentiation between native *B. terrestris audax* and non-native *B. terrestris*;
- To assess the risk of hybridization and establishment of non-native bumblebees;
- To determine the disease and parasite load of imported bumblebees and the risk of transmission to native bumblebees.

2. Research Approach

Much of the research programme in 2007 concentrated on quantifying the extent of the bumblebee importation and management in Ireland, and the population genetics of European *B. terrestris*. To establish the extent of bumblebee importation in Ireland, both numerically and geographically, 137 horticultural growers in both the Republic and Northern Ireland were surveyed in 2007 and 2009 using postal questionnaires. In addition to the 2007 survey outlined in the original project proposal, the 2009 survey allowed the estimation of trends in bumblebee importation and, more importantly, assess the impact of our recommendations on grower management of imported bumblebees. With regards to the population genetics of European *B. terrestris*, over 630 bees were sampled from 20 countries across Europe, and 2 commercial populations from the Netherlands. This represents the most extensive genetic survey of this species to date and is this first study to rigorously implement novel genetic screening protocols for cryptic species that have confounded previous studies of *B. terrestris*.

The pollination efficiency and drifting experiments were conducted in commercial polytunnels at Oak Park in 2008, as well as the field sampling of imported and native bumblebees for disease, establishment and hybridization studies. In a departure from the original proposal and to provide results directly applicable to the industry, two commercial polytunnels containing 2400 strawberry cv. Elsanta plants were established in Teagasc Oak Park. These facilities allowed the determination of optimal plant/bee density of bumblebee pollination and fruit production, the pollination efficiency of bumblebee vs. honeybees on cv. Elsanta strawberries.
and, through intensive 20-day marking experiments, the influence of plant density on drift between bumblebee hives. Field studies were conducted at six sites along the East coast. Each site contained a strawberry producer engaged in three cropping systems: glasshouses, polytunnels and open-field crops; from which 2700 wild bumblebees bees were sampled at 5 discreet distances: 250 m, 500 m 1 km, 2 km, and a 10 km control site. These wild-caught samples were then screened for disease and genetically screened to identify native, hybrid and imported bumblebees. Furthermore, to assess the degree of permeability of each cropping system to imported bumblebees, both in regards to crop pollinator management and the potential risk of negatively impacting wild bee populations, 540 pollen samples were collected from imported bumblebees at each site. By looking at the ratio of crop:non-crop pollen, this novel technique not only identifies cropping systems that allow imported bees to escape and reduced the number of pollinators on crop plants, but also the level of competition between imported and native bees for floral resources.

In 2009, the final year of the project, the disease and hybridization studies were completed and a comprehensive risk assessment exercise conducted before our evidence-based recommendations for policy and legislation were finalised. After extensive training and the provision of reference specimens from collaborators in the UK and Canada, both wild-caught bumblebees sampled in 2008 and imported bumblebees from 68 hives representing two bumblebee-rearing companies were screened for five parasites. As commercial hives were sampled at their final destination, rather than in the rearing facility as per current legislation, the ‘true’ level of infestation in situ after the stress of transport could be assessed. The wild-caught and commercial bumblebee samples were genetically screened using four nuclear and a novel mitochondrial marker, facilitating the rapid identification of native, hybrid and imported genotypes. Finally, based on the results from our drift, permeability, disease and hybridisation studies, a formal risk-assessment was conducted on imported B. terrestris using the UK Non-Native Organism Assessment Scheme. This framework quantified the risk of entry, spread, establishment and impact on the Irish economy and environment in a format amenable to policy implementation and legislative reform.

3. **Research Achievements**

Management of imported bumblebees to increase pollination efficiency.

- From our surveys in 2007 and 2009, ca. 1450 commercially imported bumblebee hives are being imported into Ireland p.a., primarily to the East and Southeast coasts.
- In 2007, less than 5% of growers were correctly disposing of their hives, but due to the targeted information campaign of this project, this increased to 28% in 2009.
- Strawberries (Fragaria x ananassa cv. Elsanta) require on 1-5 visits by bumblebees to achieve 100% pollination, increasing fruit yield by 25.95% and decreasing the incidence of fruit deformity by 47.07%.
Bumblebees, per visit, are 2.5 times more efficient pollinators than honeybees.

Plant abundance, in this study (120-180 plants/hive), did not affect the number, weight or quality of fruit i.e. crop pollination was unaffected. Therefore, one averaged sized commercial colony (one queen and 50-70 workers) should sufficiently pollinate 5,000 cv. Elsanta plants in protected crop systems.

The quantification of drift between hives and escape from areas of importation.

- Low plant abundance (120 plants/hive) increased the incidence of drift by 23% compared to high plant abundance (180 plants/hive). Bees who drifted were 5% larger and had 39% higher levels of ovary activation than non-drifting nest-mates, strongly suggesting that drift is an active process, whereby larger females seek out weaker colonies to parasitically lay eggs within. Furthermore, it confirms that drift from imported bumblebee colonies poses a significant risk for disease transmission and hybridisation with native bumblebees.
- With regards the permeability of strawberry cropping systems, 28.1%, 75.0% and 88.0% of pollen collected by imported bumblebees in glasshouses, polytunnels and field crop, respectively, is not strawberry pollen. Therefore, imported bumblebees have effectively unhindered access to interact with native bees and the utility of importing bumblebees for field grown crops is highly questionable.

Population genetics and differentiation between native *B. terrestris audax* and non-native *B. terrestris*.

- Mitochondrial markers reveal that the European subspecies of *B. terrestris* are still very closely related and statistically cannot be separated using conventional DNA barcoding methods.
- COI does contain three diagnostic sites that can reliably differentiate native Irish and British *B. t. audax* from continental subspecies.
- The faster evolving microsatellite markers reveal that inbreeding can be detected in the majority of *B. terrestris* populations across Europe, indicating recent a population bottleneck. Despite the evidence for recent inbreeding current population genetic diversity is high and comparable to levels observed 13 years ago in Southern European populations.
- There is significant recent genetic differentiation between European populations of *B. terrestris* with both commercial stocks tested being genetically distinct from wild populations.

The risk of hybridization and establishment of non-native bumblebees.

- Microsatellite and novel mitochondrial markers have been successfully developed to identify native, non-native and hybrid *B. terrestris*.
- Both mitochondrial and microsatellite markers confirm that imported bumblebees can be found up to 10 km from the site of importation.
Microsatellite markers confirm that imported bumblebees can successfully mate, produce reproductive queens, compete for hibernation sites with native bees, hibernate over winter and produce workers in the following season.

Determination of disease and parasite load of imported bumblebees and the risk of transmission to native bumblebees.

- Of the 68 commercially imported bumblebee hives screened, 1.47% contained *Apicystis bombi*, 32.29% *Crithidia bombi*, and 60.29% *Nosema bombi*, and no tracheal mites were detected. There was no statistical difference in disease incidence between the two rearing companies.
- A linear relationship was not found to exist between distance from glasshouse and the incidence of disease in wild bumblebees. However, the incidence of *A. bombi* and *C. bombi* was significantly elevated within 2 km of glasshouses, strongly suggesting that diseases are being transmitted to wild populations and being maintained at unnaturally high levels.

4. Impact of the Research

Statistics describing the extent and profile of the bumblebee importation market in the Republic and Northern Ireland have now been compiled for 2007 and 2009: i) the number, frequency, season and location of importation; ii) the crop and cropping system in which they are utilised; and iii) the attitude and self-assessed level of knowledge regarding the use and management of bumblebee hives for pollination. This will be invaluable baseline data to for any future study of the industry in Ireland and succinctly summarises the current state of the industry for stakeholders.

With respect to diseases in imported hives, the absence of tracheal mites confirms that the control measures currently implemented by commercial rearing companies are successful. However, the presence of microparasites suggests that the control measures for these pathogens implemented by commercial companies are ineffective. Microparasitic infections are known to increase with stress-induced immunosuppression, therefore, as the samples in this study were collected at the end-user (rather than at source) screening for the incidence of microparasitic infection may have been more successful as the hives have been through the stress of transport.

Imported bumblebees are maintaining microparasitic infections at higher incidences within 2 km of glasshouses, potentially having a significant negative impact on wild bumblebee populations, who themselves may act as a reservoir of disease to re-infect future populations of imported and wild bumblebees in these areas. Given the permeability of the system and the elevated incidence of microparasitic infection observed, introducing management practices
that contain imported bumblebees within the area of target crops would be both economically and environmentally beneficial. As strawberry cropping systems do not adequately contain imported bumblebees and, particularly for polytunnel and field crops, the provision of pollination for the target crop by imported bumblebees may be severely limited by the inability to keep bees on the crop and the greater attractiveness of non-crop plants. Thus, the most parsimonious management intervention for both horticultural production and conservation would be to insist that protected crop systems are ‘bee-proofed’ with netting etc., keeping imported bees on the target crop and preventing imported bees interacting with our native flora and fauna.

As confirmed by the population genetic data, island populations of *B. terrestris* are more genetically distinct than continental European populations and, therefore, they are more likely to have undergone local selection and have superior adaptations to island conditions than imported bumblebees. The genetic distinctness of commercially bred lines indicates that the companies’ breeding stock has now genetically diverged from their source populations, but rearing companies are producing colonies displaying levels of genetic diversity and inbreeding equal to that found in the wild. With increasing implementation of EU legislation regarding the introduction of non-native species and the environmental impact of biocontrol products, the potential negative impact of genetic dilution posed by imported bumblebees may be of considerable importance to commercial bumblebee exporters to the Irish and UK markets.

5. **Exploitation of the Research**

This is the first study to quantify the economic benefit of bumblebee pollination in cv. Elsanta strawberries and illustrate the impact of plant abundance on drifting behaviour in bees. The plant abundance/bumblebee hive density is relevant to the 56 countries worldwide currently importing commercially reared bumblebees for strawberry pollination, particularly at the cost:benefit ratio for investing in pollination can now be accurately calculated for this crop. Furthermore, the permeability of glasshouses and polytunnels, and the striking lack of time spent on open field crops, suggest a change in management practices regarding containment of pollinators in protected crop systems and questioning the current practice of investing in imported bumblebee pollinators for non-protected crops.

The significance of microparasites in commercially imported colonies and transmission of parasites to wild populations cannot be understated. Although current EU and national legislation focuses exclusively honeybee pathogens, this is under constant review and the licensing of bumblebee colonies based on the presence/absence of honeybee disease will cease in the near future. Once this occurs, there may be severe restrictions placed on the importation of non-native bumblebees and responsibility for licensing bee importation may devolve to national governments. If this occurs, there will be an immediate negative impact on
Irish horticultural production and competitiveness due to lack of suitable pollinators and consequent reduction in yields. Additionally, there may be increased costs involved in state-controlled licensing and independent disease screening of imported hives.

Finally, the development of a novel mitochondrial genetic marker to reliably differentiate between native Irish and non-native *B. terrestris* will be an invaluable tool in future commercial breeding practices and environmental impact assessments involving imported bumblebees. The suite of microsatellite markers employed in this study, although not novel, have affirmed their utility in differentiating native, hybrid and non-native genotypes with Ireland. Further refinement of these genetic tools would facilitate rapid and economical genetic high-throughput screening practices of significant use to bumblebee-rearing companies and environmental protection agencies.

6. **Summary of Research Outputs**

(a) Intellectual Property applications/licences/patents
1. Nil.

(b) Innovations adopted by industry
1. Correct disposal of hives.

(c) Number of companies in receipt of information
1. Syngenta.
2. Koppert.

(d) Outcomes with economic potential
1. Confirmation that bumblebees are 2.5 times more efficient than honeybees for strawberry pollination, therefore should become the industry’s standard pollinator of this crop in Ireland.
2. ‘Bee-proofing’ protected crop systems to keep imported bumblebees on target crops and maximise pollination.
3. Recommended hiver/crop plant density in strawberries should reduce excessive investment in imported pollinators.
4. Reduction in the number of hives imported for field-grown crops where non-crop plants are significantly favoured by imported bees.
5. The high incidence of microparasitic disease in commercial colonies and the negative environmental impact of imported bumblebees on native bees may lead to changes in legislation restricting their movement within the EU. This may lead to a decline in bumblebee importation, depriving Irish protected crop growers of a valuable pollinator.
Outcomes with national/ policy/social/environmental potential

1. The confirmation that bumblebees, rather than honeybees, are highly evolved pollinators of strawberry should be used to highlight the role of the other 100 species of bee present in Ireland in agriculture. Compared to pollination, honey production is a minor facet of the monetary contribution of bees to Irish agricultural production. Thus, the historic emphasis and investment in honey production may have been misplaced when compared to the potential economic gains provided by research and investment in modern horticultural and biofuel crop pollination practices.

2. The UKNNRA Scheme provides a framework for the assessment of the risks posed by any non-native organism to species, habitats or ecosystems in all or part of the island of Ireland. The risk assessment successfully completed as part of this project highlights the utility of this framework for summarising knowledge, identifying uncertainties, prioritising future research and recommending management options for other non-native organisms in Ireland.

3. The evidence for disease transmission, hybridisation and establishment of non-native bumblebees in Ireland is now compelling. The consequences of maintaining the status quo regarding licensing and lack of independent screening without further research is to endanger all native populations of pollinators and leave open an avenue for future pests and pathogens to enter Ireland.


Scientific abstracts or articles including those presented at conferences


(h) National Report 0
(i) Popular non-scientific publications

3. Horgan F (2007) Report on bee collecting trips to Poland, Denmark and Isle of Man (RMIS 5633); Teagasc, Oak Park.

(j) Workshops/seminars/open days at which results were presented (excluding those in (g))


7. Permanent Researchers

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8. Researchers Funded by RSF

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9. Postgraduate Research

Total Number of PhD theses: One

‘Genetic Analysis of Commercial and Native Bombus’. Mr. Sergio Moreira, School of Science, Institute of Technology Carlow.

Please include authors, institutions and titles of theses and submission dates. If not submitted please give the anticipated submission date.
Total Number of Masters theses: 

Please include authors, institutions and titles of theses and submission dates. If not submitted please give the anticipated submission date.

10. Project Expenditure

Total expenditure of the project: €283,979.04

Total Award by RSF €299,901.00

Other sources of funding (specify) €

1. 
2. 

Breakdown of Total Expenditure

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11. **Future Strategies**

Given the marked declines in honeybee populations and the 30% of bee biodiversity is currently under threat in Ireland, the economic and ecological contribution of native bee pollination to horticultural production in Ireland remains largely unknown. Future research should focus on:

1. Determining the extent of pollination limitation in Irish horticultural produce i.e. identify areas of poor/reduced yield that are lacking sufficient pollinators.
2. Quantifying the increase in yield (pollination efficiency) attributable to managed honeybees, native wild bees and managed bumblebees for horticultural crops that are known to require significant insect-mediated pollination.
3. Efficacy of agri-environment schemes currently utilised in Europe for promoting pollinator diversity and abundance in areas of horticultural production.
4. Developing predictive (e.g. GIS-based) tools to identify regions with poor landscape/invertebrate diversity. Where native bees are lacking, develop management plans for short-term supplementation of managed bees and longer-term management plans integrating agri-environment schemes promoting native pollinator abundance and diversity.

The UKNNRA Risk Assessment document completed as part of this project has summarised and incorporated the most up-to-date data on the risks associated with bumblebee importation. Furthermore, the assignment of uncertainties to each risk factor has focussed and prioritised future areas of research to reduce uncertainty in future assessments:

1. Genetic and morphometric survey in Ireland comparing pre- vs. post-introduction specimens and imported vs. native specimens.
2. Independent disease screening of imported colonies using improved genetic techniques and evaluating the impact on native bumblebees and honeybees.
3. Establish the extent of establishment within and beyond horticultural areas, including focused surveys on bees foraging out of season in winter: are they imported, native or hybrid bumblebees?
4. Reproductive behavioural ecology and rates of hybridisation, including pheromone biology and dispersal rates.
5. Improved control methods of preventing sexuals from escaping from commercial colonies.
6. Study the extent of competition in the field.
7. Research efficacy of control measures limiting the escape of imported bumblebees.
8. Research efficacy of eradication measures.
12. **Industry Collaboration**

The International Biocontrol Manufacturers Association facilitated dissemination of guidelines to stakeholders for disposal of imported hives. In terms of disease screening and population genetics of *B. terrestris*, representatives of commercial bumblebee-rearing companies, Koppert and Syngenta, provided qualitative data and expressed significant interest in expanding the research, particularly with reference to rapid genetic-based diagnostic of viral and micoparasitic infection.

The Wexford Strawberry Growers Co-operative, the Irish Strawberry Growers Association, the Dept. Agriculture and Rural Development NI and private horticulturists north and south of the border contributed to the all-Ireland survey in 2007 and 2009, and facilitating *in situ* experiments at the six sites involved in the disease, permeability and hybridisation studies.

In terms of conducting and producing a formal invasive species risk assessment, Dr. Martin Damus of the Canadian Food Inspection Agency and Dr. Juliet Osborne of Rothamsted Research U.K. were instrumental in providing expert guidance in the production of the UKNNRA document.