**HL0191 - Minimising pesticide residues in strawberry through integrated pest, disease and environmental crop management**

**Aim of Initiative:**

The overall aim of the proposed project is to develop alternative, sustainable, non-pesticidal methods for managing Botrytis, mildew, blackspot, aphids, blossom weevil and capsid bugs on strawberry so greatly reducing (by >50%) pesticide use and eliminating the occurrence of reportable pesticide residues on harvested fruit. The methods developed for the individual pests and diseases will be combined with existing non-chemical methods for other pests and diseases in an overall Integrated Pest and Disease Management (IPDM) system, and this will be tested and refined in commercial strawberry production over 2 seasons. A further important aim is to improve the environmental acceptability of strawberry growing so that it is better harmonised within the rural landscape. The aims of this initiative are, thus, fully consistent with those of the EU Thematic Strategy on the Sustainable Use of Pesticides by encouraging pesticide-free crop farming and Defra’s objectives of improving the environmental sustainability of agricultural and horticultural production.

**Commercial and Technical Background:**

Strawberries are very susceptible to many pests and diseases but the most important that cannot currently be controlled by non-pesticidal means are *Botrytis*, mildew, blackspot, European tarnished plant bug, strawberry blossom weevil and aphids. The crop cannot be grown economically unless these pests and diseases are controlled effectively. The UK industry and other producers rely on pesticides for this purpose and thus strawberry crops are intensively treated with pesticides. Intensive use of pesticides, including of the anticholinesterase insecticides chlorpyrifos and pirimicarb, has a negative impact on the environment and compromises the sustainability of production. Residue surveillance shows that approximately 60% of UK produced strawberries contain pesticide residues with multiple residues in 25% of samples. The occurrence of such residues undermines consumer acceptability of the product.

The UK industry wants to reduce the environmental impact and improve the sustainability of strawberry growing. Multiple retailers want to eliminate reportable residues from strawberries to improve consumer trust. UK strawberry growers want to work towards this difficult goal, minimising the incidence of residues and improving the environmental acceptability of UK strawberry production. There is strong market demand, led by consumer expectations, to eliminate the occurrence of residues.

Strawberries are adversely affected by rain and, to meet the requirements of major multiple retailers for reliable, season-long supply, the crop is now mainly grown under protection. Humid conditions in the protected environment are favourable to *Botrytis* which is the major cause of post-harvest fruit rotting which causes serious yield losses. Poor shelf-life of infected fruit reduces repeat buying. Protected cropping has resulted in a greatly increased risk of mildew. Blackspot has become endemic; though the disease is less of a threat in protected cropping if the foliage remains dry, crops are treated routinely with fungicides to prevent outbreaks. Capsid bugs, blossom weevil and aphids are common and often abundant and damaging pests wherever strawberries are grown. Routine applications of insecticides...
are often made for control, disrupting biocontrol programmes for other pests.

The commercial and technical background to each of the diseases and pests to be studied in the project are described under headings below:

**Powdery mildew**

Powdery mildew, caused by *Podosphaera aphanis* Braun & Takamatus (formerly *Sphaerotheca macularis* (Wallr.:Fr.) Lind syn. *S. humuli* (DC.) Burrill), is a serious disease on strawberries (*Fragaria x ananassa*) (Miller *et al.*, 2003; Blanco *et al.*, 2004; Amsalem *et al.*, 2006), particularly late-season and everbearing types, and strawberries grown under protection in glasshouses or polytunnels. It can infect leaves, leaf petioles, flower trusses, flowers and fruit. Serious damage to the foliage results in reduction of photosynthesis due to dense mycelium coverage, which can lead to necrosis and eventual defoliation (Maas, 1998). Yield losses may also be inflicted due to infection of flowers and fruit, organs which are susceptible to infection at all stages of development. Affected flowers may be deformed, produce low levels of pollen or wilt and die, while infected green fruit fail to ripen and infected ripe fruit remain soft, have a shortened shelf life and possess small seeds (Spencer, 1978).

Because of the lack of cultivars with durable resistance, strawberry powdery mildew is currently controlled by routine sprays of fungicides. However, it is difficult to control the disease with the fungicides available, especially in everbearers because of their long cropping period and the difficulty of scheduling spray applications that comply with minimum harvest intervals during regular harvesting. In addition, increasingly both June-bearing and everbearing strawberries are being grown under protection, which has led to more severe epidemics than in open field conditions (Xiao *et al.*, 2001). The under-protection production allows growers to schedule their harvests to coincide with periods of market demand and may also help to reduce grey mould, fruit rots and root rots (Freeman *et al.*, 1997; Legard *et al.*, 2001; Xiao *et al.*, 2001).

However, without the inhibitory effect of rain on conidia germination, protected crops tend to have more powdery mildew infection (Legard *et al.*, 2001; Xiao *et al.*, 2001). Powdery mildew is an increasing problem on protected strawberry crops in the UK. Long periods at around 20°C and the high relative humidity in tunnels provide favourable conditions for *P. aphanis* (Amsalem *et al.*, 2006). Under protection, pesticides may take longer time to dissipate because the rain wash-off does not take place. Thus label recommendation for open field production may not be applicable to under protection production.

**Botrytis**

Grey mould (*Botrytis cinerea*) is a serious disease of strawberry that causes significant yield losses through fruit rotting (Sutton, 1998; Berrie, 2004). The disease can be found in most crops in most years. Losses in unprotected crops in wet years can be devastating. Infection occurs primarily through flower parts and growers currently apply fungicides routinely at 7-10 day intervals during flowering to prevent fruit botrytis. Losses in protected crops are generally less than in open-field crops but visible botrytis still develops in some fruit post-harvest causing rejection of punnets. Annual losses to botrytis are unpredictable and consequently growers still apply protectant fungicides routinely. A 2006 survey showed that fungicides applied for botrytis control (e.g. chlorothalonil, fenhexamid, iprodione, tolylfluanid) accounted for over 50% by weight of fungicide active ingredients applied to strawberry
Walker, 2007). Residues of botrytis fungicides are frequently found in marketed strawberry fruit. Although levels are almost always less than the MRL, the presence of any detectable residue raises consumer concerns. For example, Marks & Spencer are working towards just two categories of fresh produce, organic and residue-free. An alternative approach for control of fruit botrytis is needed so that control of this disease does not have adverse consequences for the environment and that consumer requirements for minimum residues are met.

Blackspot

Blackspot is caused by the fungus Colletotrichum acutatum and can attack all parts of strawberry plants, including roots but is primarily a rot of ripe fruit, which under favourable conditions of warm wet / humid weather, can cause significant losses in the field and post harvest during marketing. Losses in 2002 in some commercial crops of the June–bearer Elsanta were up to 38% of fruit infected at harvest. Losses in everbearer crops, however, can be far higher with as much as 80% of fruit infected with almost total crop loss where control becomes impossible.

Unlike botrytis and powdery mildew, blackspot is not well controlled by applying fungicides at set timings in conjunction with disease monitoring as there are currently no very effective fungicides for controlling the problem. Furthermore, the disease is often symptomless until the fruit starts to ripen, thus fungicides may have to be used repeatedly including during harvest. The 2001 survey of pesticide usage in soft fruit in Great Britain (Garthwaite & Thomas, 2001) showed that strawberries receive an average of 5 applications of fungicide per annum, 46% of which is targeted against blackspot. Such an approach to the problem is not sustainable and results in fungicide residues in fruit at harvest. The incidence of blackspot is much less in crops grown under protection, however not all strawberries are grown in this way and not all crops are protected throughout the season, giving ample opportunity for blackspot to infect and spread.

C. acutatum can survive in crop debris in soil for 9–12 months or more depending on soil conditions (Eastburn & Gubler, 1990), but once it decays the infection risk goes. Soil sterilisation is currently recommended as part of the integrated approach to eliminate the risk of blackspot in new crops. Soil fumigation with methyl bromide / chloropicrin mixtures was shown to be effective in eliminating C. acutatum in trials in USA (Gubler et al., 1988). Basamid was less effective and efficacy of other chemical sterilants and biological methods of soil disinfestation (eg incorporation of mustard) is not known. Infected planting material is considered to be the main means of introducing C. acutatum into new crops and areas, but the fungus has a wide host range including many other fruit crops such as apples and cherries, and more recently raspberries and blueberries, ornamentals and weeds (McLean & Roy, 1991; Smith, 2001; Freeman et al., 2001). Exactly how important these other hosts are as sources of inoculum is not clear. The loss of methyl bromide as a soil sterilant could increase the risk of blackspot for strawberry crops by better survival of C. acutatum on debris in soil and by increasing weed numbers, which are a potential source of inoculum, particularly when treated with herbicides (Anon, 1979). Recently, at EMR the incidence of Colletotrichum on cherry and apple has increased considerably. A clear understanding of the importance of weeds, other fruit crops and ornamentals as sources of inoculum would enable appropriate control measures to be applied.

European tarnished plant bug

In late season strawberry crops L. rugulipennis feeding in flowers and on green fruits can cause up to 80% crop loss, rendering production uneconomic (Cross, 2004). In conventional crops the pest is controlled by sprays of broad-spectrum insecticides in mid and late summer, the organophosphorus insecticide chlorpyrifos being the most effective and frequently used. June bearer strawberries typically receive 2-4 applications of insecticides per annum and everbearers often receive more, with 6 or more applications being required where there are successive attacks by European tarnished plant bug and western flower thrips. There is a high incidence of chlorpyrifos residues in strawberry fruits caused
mainly by these applications. Neonicotinoids and other modern insecticide groups are only partially effective against *L. rugulipennis*, and insect growth regulators are totally ineffective. In organic crops *L. rugulipennis* causes high levels of damage because the insecticides available are inadequate and of short persistence. The economic threshold for *L. rugulipennis* has been estimated as 1 capsid per 40 plants, a very low level which is difficult to detect by visual inspection (Jay *et al.*, 2004). Crop invasion by the pest is sporadic and unpredictable, and, in the absence of effective control measures, severe economic losses are caused at low population densities which are difficult to detect in normal crop inspections. Application of broad-spectrum pesticides to control *L. rugulipennis* is an important barrier to the implementation of biocontrol systems in strawberries. Effective biocontrol methods have not been developed for *L. rugulipennis*. The effects of different predators and parasitoids on *L. rugulipennis* populations were reviewed by Cross *et al.* (2001). Work in Hort LINK project HL0184 is developing pheromone traps for monitoring capsid bug populations including for *L rugulipennis*. These traps should provide important assistance to growers in monitoring the pest, but innovative control strategies are still needed.

**Strawberry aphid**

Several species of aphids are pests of strawberry, but the strawberry aphid, *Chaetosiphon fragaefolii*, is the most common and troublesome and is the focus of the proposed research because current biological control methods for it are unsatisfactory. The strawberry aphid can be a serious pest on strawberries, especially those grown in the elevated temperatures under polytunnels. The aphid produces honeydew in which sooty moulds grow, leading to downgrading of fruit. It is also a vector of three serious strawberry viruses (Shanks, 1981); infection with a complex of aphid-transmitted viruses can reduce yield (Craig, 1957; Freeman & Mellor, 1962). Current research at EMR (Defra HH3229) is investigating the association between aphid feeding and subsequent virus spread within the crop. The aphid is currently commercially controlled by applications of insecticides in spring which may lead to residues in fruit; pirimicarb, used to control aphids is one of the most frequently detected residues in fruit. Some of the available insecticides are also incompatible with IPM programmes as they are toxic to predatory mites, including *Phytoseiulus persimilis*, which is used as a biocontrol agent for the two-spotted spider mite, *Tetranychus urticae*, and to predatory insects which are generalist predators in the crop. Methods that enhance populations of predators and parasitoids in the crop should reduce reliance on aphicide applications to reduce *C. fragaefolii* populations and also increase biocontrol of other pests. Many species of predators and parasitoids occur naturally in crops and surrounding vegetation. Manipulation and management of field margins to provide attractive vegetation, both in terms of refuges and alternative food sources should increase the abundance of beneficial species in the crop area (e.g. Fitzgerald and Solomon, 2004). The use of lures containing plant derived semiochemicals may attract beneficials into the crop. This has been evaluated in some crops (e.g. James, 2003b in hops) but has not been evaluated in strawberry. The use of selective insecticides post harvest would reduce overwintering populations of *C. fragaefolii* and allow beneficial species present in the crop to survive, so enabling early season biocontrol of a range of pests (e.g Easterbrook *et al.*, 2006; reviewed by Cross *et al.*, 2001). Early introductions of mass produced predators or parasitoids could also reduce populations of aphids, but the species used would need to be able to survive cool night temperatures.

**Strawberry blossom weevil**
Strawberry blossom weevil, *Anthonomus rubi*, is a serious pest of strawberry and a minor pest of raspberry and blackberry in commercial production in the UK. Females lay eggs singly in flower buds then sever them. Yield loss depends on the plants yield compensatory capacity and the number of flowers remaining in relation to the yield potential of the plants crown and root system. Losses can be severe if the pest is not controlled effectively especially in highly valuable first year crops which have relatively few flowers. The weevils migrate from crop to crop moving into those crops that provide flower buds for further egg laying, so causing damage into late summer. Severity of attack is unpredictable. Control relies on a pre-flowering spray of chlorpyrifos or thiacloprid to control adults, often applied routinely. Alternative non-chemical control methods for the pest are needed so that control of this pest does not have adverse consequences for the environment or consumers.

The Problem/Opportunity:

Dependence on pesticides in strawberry production, especially during flowering and fruit development and close to harvest, needs to be reduced. There is an opportunity to develop alternative, non-pesticidal approaches for the key pests and diseases of strawberries grown under protection and then combine them in an integrated management programme.

Scientific Background:

**Powdery mildew**

1. There is much published information on the biology and epidemiology of powdery mildew, particularly during the growing season:
   - The optimal environmental conditions for conidial germination and further growth ranged between 15 and 25°C with relative humidity (RH) higher than 75%, but less than 98%. Conidia survival declined over time, but a certain percentage of conidia remained active after 5 months incubation in a range of temperatures.
   - The rate of conidial germination was significantly higher on young leaves than on older leaves. Sporulation at 70–75% RH was similar to that at 80–85%, but greater than that at 95% RH. The shortest time from inoculation to appearance of the first disease symptoms was only 4 days at 20°C.
   - In general, the environmental conditions required for germination and dispersal of powdery mildew are conducive to disease progress under strawberry production conditions.
   - A simple prediction system has recently been developed with funding from HDC project SF 62 (University of Hertfordshire and CSL); this system uses hourly temperature and humidity as input (Dodgson *et al.*, 2007). Preliminary evaluation results suggested that management strategies based on the predictions may result in satisfactory disease control but with less fungicide input.
   - There is a lack of genetic variation among sampled mildew isolates from Italy and Israel (Pertot *et al.*, 2007), and the UK as shown recently at EMR. Furthermore, there are no clear race-specific relationships.
   - There is no information on susceptibility of powdery mildew in relation to nitrogen levels.
2. There is uncertainty on the relative importance of overwintering mechanisms:
   - Powdery mildew is believed to overwinter as mycelium on living infected leaves (Peries, 1962). However, recent mild winters have negated the importance of mycelia on infected leaves in the autumn as primary inoculum for powdery mildew in the spring because infected green leaves in autumn continue to grow during the warm winter and become senescent by the early spring. Sites with chasmothecia (sexual reproduction bodies, producing ascospores) of powdery mildew tend to have mildew about 4-8 weeks earlier in the spring than sites without the chasmothecia.
   - Strawberry mildew is heterothallic and chasmothecia are often produced (Peries, 1962; Maas, 1998; Nakazawa & Uchida, 1998; Berrie et al., 2002) but their role in the life cycle of this disease as perennation bodies has not yet been determined. However, recent research at EMR and elsewhere has demonstrated that ascospores can remain viable over the winter and infect plants in the spring (Xu et al., 2007; Hall et al., 2007).
   - With changing planting systems, particularly different types of initial planting materials, the relative importance of different sources of primary inoculum clearly deserves urgent investigations. Recent work showed the possibility of carry-over of inoculum from planting material.

3. One of the potential drawbacks of molecular detection methods, based on the detection of DNA alone is that it is possible to get positive results from dead or non-infectious pathogen, due to the persistence of the relatively stable DNA molecule. Whilst in most cases this gives an indication of ‘recent presence’ due to the environment being a hostile place for DNA to remain (as DNA will degrade rapidly), especially in dead or decaying tissue it is still problematic. Methods based on the detection of RNA offer a potential solution to this problem. In a living cell RNA is constantly being ‘turned over’, transcribed to enable protein production and then broken down by the cell. Furthermore RNA is very labile and as such, in the event of cell death is degraded very rapidly. Molecular methods (e.g. Loeffler et al., 2001; Bentsink et al., 2002) can be designed to exploit this phenomenon, and by using these RNA specific assays it is possible to get detection of live pathogen only. One such method NASBA (nucleic acid based sequence amplification) can be used to amplify only from RNA template present in the sample. Following amplification using NASBA the products of amplification can then be monitored using molecular beacons – enabling real-time detection and hence accurate quantification, not only of the presence of the pathogen but also its viability.

4. Disease management relies almost exclusively on routine fungicide applications:
   - Fungicide dissipation under protection is generally not known as application of most fungicides still follows the guidelines for open-field production.
   - It is generally accepted that BCAs alone are not likely to achieve effective control of diseases in commercial production and may need to be integrated with reduced doses of fungicides or natural products.
   - Thus, knowledge on the survival and colonisation of strawberry (old and new tissues) by BCAs in the presence or absence of fungicides is important for developing strategies of using BCAs.

5. There is a lack of knowledge about plant susceptibility in relation to nitrogen:
   - Factors affecting tissue susceptibility vary with mildew species and its host.
   - Some important factors are osmotic pressure (Schnathorst, 1959) (susceptibility to mildew increases with increasing water content of the tissue) and starch / sugar content (Schoeman et al., 1995).
   - Host nutrition also has an impact on susceptibility to mildew. Nitrogen has the greatest effect on susceptibility, higher nitrogen equals greater mildew susceptibility. Young leaves and soft growth of various species are reported to be more susceptible to powdery mildew fungi. The effect of nitrogen on plant diseases is complex and can be inconsistent (Walters & Bingham, 2007). There is evidence that nitrogen supply affects disease though change in content of nitrogenous
substances in leaves, rather than as a result an effect on canopy growth and microclimate (Neumann et al., 2004). In contrast to nitrogen, tissues deficient in potassium tend to be less mildew susceptible (Cole, 1964).

Botrytis

Risk of latent B. cinerea in young plant material

Young strawberry plants raised in module trays at high density are sometimes affected by visible grey mould. Others produced as runners and kept in cold-store until required often have moribund petioles and leaf debris at planting that is susceptible to infection by B. cinerea. B. cinerea is one of several fungi that can cause plant deterioration in cold storage (Maas, 1998). B. cinerea is tolerant of cold and can develop and cause crop damage even at 0°C. Spore germination on grapes took 8 days at 0°C compared with < 1 day at 20°C; the amount of fungal growth after 12 days at 0°C equalled that after 1 day at 20°C (Ish-Shalom et al., 2007). It is likely that some apparently healthy plants have symptomless (latent) B. cinerea infection. The occurrence of latent B. cinerea in strawberry planting material requires investigation.

Epidemiology of strawberry fruit rot

The occurrence of latent infection by B. cinerea in strawberry leaves was first described in Canada (Braun & Sutton, 1988). B. cinerea primarily infects young strawberry leaves, remains latent in epidermal cells until leaves senesce, and then sporulates in tissues when they die (Sutton, 1998). Dispersal spores produced from mycelium in dead strawberry leaves are considered the primary source of inoculum leading to fruit rot in strawberry crops; rotted fruit is a secondary source, and can be particularly important in everbearer crops.

In previous Defra-funded research, EMR have developed a weather-based model that accurately predicts flower infection and subsequent fruit rot by B. cinerea in field-grown strawberry (Xu et al., 2000). This model was implemented as a stand-alone computer programme, called BOTEM (Botrytis East Malling); BOTEM only needs hourly temperature and humidity to predict disease risk. BOTEM was evaluated in the UK for three years at two sites in collaboration with Fruit Advisory Services Team (FAST). In all years, management strategies based on BOTEM resulted in comparable disease control but with considerable less fungicide input. From this evaluation project, we have developed an overall management programme taking into account the disease risks and % flowers opened. The essence of this strategy is that sprays are applied curatively according to the BOTEM risk, starting from first flower when the predicted fruit infection has reached a threshold of 10%, but if no sprays have been applied by 30% flowering or more than 14 days has elapsed since a spray was applied during flowering, then a routine spray of a chosen fungicide is applied.

BOTEM has recently been evaluated together with several other forecasting systems for strawberry grey mould in the Netherlands, Belgium and Ireland. These evaluations showed that BOTEM consistently performs the best (Piet Creemers, Kieron Bradley, pers. comm.). BOTEM is being used successfully by consultants in these countries, and scientists and consultants in Norway and Germany have also started to evaluate BOTEM in 2007. But it is not yet adopted commercially in the UK. This is mainly because of the lack of funding to promote and demonstrate the system on a larger scale to growers. Contrary to the current situation, when BOTEM was developed many growers were not familiar with using on-site weather data and computer models to predict disease risk. Growers have also not yet perceived the benefits of using the system to reduce the risk of pesticide residues. The key parameter
that correlated closely with flower infection was very high humidity. Infection did not correlate closely with temperature or leaf wetness duration.

As BOTEM only needs temperature and humidity as inputs, it should be easier to adapt the system for use on protected crops. Furthermore, in developing BOTEM we showed that inclusion of daily inoculum concentration (Xu et al, 2000) did not improve the model performance sufficiently enough to justify the extra cost and difficulties for growers to obtain such information. Nevertheless, the BOTEM model requires further testing to determine its validity on protected crops; the results can then be used to modify the model parameters if necessary. Theoretically, because crops are protected from rain, the frequency of periods of a very high humidity in polythene tunnels should be less than in open-field, with a consequent reduced need to apply botrytis fungicides during flowering. This system is inexpensive and simple to use: only temperature and humidity are needed (a dual sensor is about £50) and most growers have already got computers.

Application of molecular methods to botrytis research

TaqMan PCR provides a very sensitive and specific test for latent B. cinerea in plant tissues. We propose to use this method to check for overwintering latent infections in strawberry crown tissue. TaqMan PCR also opportunity to examine whether thresholds of latent infection in young strawberry plants can be set that relate to risk of subsequent botrytis development. However, experience gained in HL0166 indicates that the testing of a large number of samples would be required to test this hypothesis, leading to a disproportionate cost on a small aspect in the current broad proposal. We shall not therefore attempt to determine threshold levels.

The discovery of long-term, symptomless systemic infections by B. cinerea in some crop species could be relevant with regard to strawberry fruit rot. In this project we propose to investigate the occurrence of latent B. cinerea within crown and/or the first emerging leaf after removal of runners from cold store.

Tracking of characterised B. cinerea isolates in a tomato crop showed that within-crop sporulation (endogenous spora), rather than introduced (exogenous) air-spora, was the major source of new botrytis lesions (Decognet et al., 2007). It suggests that early-season disease control could have a major impact on epidemic development for a polycyclic disease such as B. cinerea. The cost of collecting, cleaning and characterising a sufficiently large numbers of B. cinerea isolates necessary to determine the source of isolates affecting strawberry fruit is outside the scope of the current proposal. However, we propose to investigate measures to reduce inoculum early in epidemic development.

Fungicidal control

Several new botrytis fungicides with novel modes of action (i.e., new fungicide groups) have become available in recent years, including boscalid + pyraclostrobin (Signum), fenhexamid (Teldor), mepanipyrim (Frupica), pyrimethanil (Scala), cyprodinil + fludioxonil (Switch); all of these products are now being used on strawberry. Some older botrytis fungicides are currently no longer available (eg carbendazim, dichlofluanid), or may be less effective (eg iprodione) due to the occurrence of fungicide-resistant pathotypes. Information on the efficacy of currently-available fungicides to suppress sporulation on dead strawberry leaves is limited, with the last study in 2000 (Berrie, 2000). Recent work
in the USA indicated that fungicides differ in their ability to control latent *B. cinerea* in grape berries (Zitter & Wilcox, 2007). There is no information available on the efficacy of currently available fungicides to reduce or eliminate latent *B. cinerea* in strawberry leaves. We propose to determine the effect of newly available botrytis fungicides on young plants around planting and pre-flowering in order to reduce inoculum production during flowering.

**Potential novel methods for control of botrytis**

We considered the potential of ozone, UV light, electrolysed water and biocontrol agents vectored by bees for control of botrytis on strawberry. After literature reviews and consideration, it was decided that there was sufficient evidence of useful disease control using BCAs vectored by bees to justify inclusion in the project, but not of the other topics.

**Use of bees to deliver BCAs to flowers**

Several biocontrol products with activity against *B. cinerea* have been developed including Binab T Vector (*Trichoderma harzianum* and *T. polysporum*) and Serenade (*Bacillus subtilis* strain QST 713). These two products are currently being assessed by PSD for registration in the UK for use on strawberry. Biocontrol products are usually applied to crops as high volume sprays. However, there is increasing interest in the use of bees to vector biocontrol agents directly to flowers (Dedej et al., 2004; Maccagnani et al., 2005; Shafir et al., 2006). Binab T Vector is a formulation specifically for bee dispersal and is available for use in strawberry crops in Sweden. This product and method of application has not been evaluated under UK conditions. *Bacillus subtilis* was shown to be dispersed to blueberry flowers by honey bees (Dedej et al., 2004), and is claimed to control strawberry botrytis fruit rot when applied regularly during flowering (www.Agraquest.com), but this BCA has not been tested for control of strawberry botrytis fruit rot using a bee dispersal system to our knowledge.

For botrytis-susceptible fruit crops such as strawberry, this would appear to be an ideal method for dispersing a BCA directly to the most susceptible plant parts. Moreover, bees are generally active in the crop (and so able to disperse biocontrol agents) when it is in flower and most susceptible to infection. Honey bees and bumble bees have both been shown to be capable of delivering formulated biocontrol agents to strawberry flowers and provide control of botrytis fruit infection equivalent to a fungicide programme in open-field strawberries in the USA and Israel (Kovach et al., 2000; Sutton et al., 1997; Shafir et al., 2006).

At present, there are no biocontrol products registered for use on strawberry for control of *B. cinerea* in the UK. There is no information on the efficacy of BCAs dispersed by bees for control of strawberry botrytis fruit infection in protected crops in the UK. If bee-vectored BCAs are effective in controlling botrytis fruit rot, this could provide a very significant new tool in an IPM programme leading towards zero-residue fruit in the UK. Currently EMR is evaluating BCAs (via conventional application) for control of *B. cinerea* on strawberry with funding from PSD.

There is already considerable use of bumble bees, and to a lesser extent honey bees, in protected strawberry crops in the UK to improve flower pollination. However, there is uncertainty over the long-term legality of using imported bumble bees. While the environmental benefits of reducing the number of imported bumble bee colonies (a European sub-species not native to the UK) are currently unknown, it was clearly shown in field trials that imported bumble bees (*Bombus terrestris dalmatinus*) are out-competing the UK native sub-species (*B.t. audax*) (Ings et al., 2006). Natural England’s view is that the
escape of commercial bumble bees contravenes the Wildlife and Countryside Act (1981), making it an offence to allow to escape, or to allow the release of 'any animal which is of a kind which is not ordinarily resident in or is not a regular visitor to Great Britain in a wild state'. As such, any release of the currently marketed commercial bumble bees into the wild may be an offence under the act.

There is also uncertainty over whether honey bees in the UK would show sufficient activity in cool, humid weather, conditions conducive to flower infection by *B. cinerea*. It has been suggested that a mixture of bumble bees and honey bees can have a synergistic effect in terms of BCA dispersal under different weather conditions and the reliability of associated botrytis fruit rot (Yu & Sutton, 1997).

We propose to investigate the use of two BCAs (Binab T Vector and a formulation of *B. subtilis*) vectored by honey and/or bumble bees for control of botrytis fruit rot in strawberry. In year 1, we propose to use both types of bee at one experimental site, while clarification and consortium agreement on which type of bee to work with in subsequent years is sought.

**Blackspot**

The biology and epidemiology of *C. acutatum* has been well researched in many countries the main results of which are summarised below.

- **Blackspot** is caused by the fungus *C. acutatum* and can attack all parts of strawberry plants, including roots but is primarily a rot of ripe fruit. The fungus can often remain symptomless and not appear as visible infection until harvest. All current strawberry cultivars are susceptible to infection.
- *C. acutatum* has a very wide crop plant host range including many fruit crops and ornamentals and weeds. It also can remain symptomless on many of these crops particularly weeds. These may act as sources of inoculum (Hildebrand & Jensen, 1991; McLean & Roy, 1991; Smith, 2001; Freeman et al., 2001).
- Symptomless infected planting material is considered the main means of introduction into new areas and onto new farms (Legard, 2000; Gubler, 1998; Browne et al., 1992).
- The fungus can survive on crop debris in soil and on the soil surface. Length of survival time depends on conditions – longer survival under cool, dry conditions (Eastburn & Gubler, 1990).
- *C. acutatum* spore masses are sticky and slimey and readily adhere to surfaces of anything that comes into contact, eg birds, animals, pickers hands, clothes, machinery and other equipment and can spread within and between crops in this way (Yang et al., 1990).
- Water splash (rain or irrigation) is responsible for localised spread. Trials in the USA have shown that water splash spread is greater on plastic mulch and bare soil and is reduced by straw mulch (Yang et al., 1990; Madden et al., 1993).
- Blackspot is a warm, wet weather disease with optimum temperatures of 25-30°C. Conditions of optimum temperature and 13 hours wetness can result in more than 80% fruit infection. Spore dispersal and subsequent infection of fruit can occur with <15 minutes duration of rain (Santos et al., 2001).
- The blackspot fungus can increase on the leaf surface of strawberry without showing any symptoms and this explains why blackspot can suddenly appear on fruit without prior plant symptoms (Leandro et al., 1999).
- If climate change results in higher temperatures in spring and early summer, and with rain or irrigation water to disperse conidia, there could be significantly increased development of *C. acutatum* on plants prior to protection, increasing the risk of blackspot on fruit under protection. Field crops will be at even greater risk.
- Phosphorus and potassium have no effect on blackspot incidence, whereas high nitrogen fertiliser (particularly ammonium fertilisers) increases blackspot severity (Smith, 1987; 1989).
• Calcium salts, particularly calcium sulphate, are reported to reduce fruit rot incidence but recent experiments in the UK did not support these results (Smith & Gupton, 1993)
• Herbicides such as paraquat and glyphosate (Roundup) stimulate C acutatum- infected tissue to sporulate so can considerably increase disease spread. The effect of other herbicides is not known, but death of plant material is likely to result in sporulation of C. acutatum (Biggs, 1995)
• Successful control of blackspot is dependent on an integrated approach, combining soil sterilisation, healthy planting material and use of protective covers, with applications of fungicides also targeted at powdery mildew and botrytis.
• Soil sterilisation and protective covers are key to the integrated approach. Experience in other EU countries where soil sterilisation is not permitted indicates a significant increase in blackspot incidence in strawberry crops.
• Molecular methods for characterising populations of Colletotrichum species have been well established.

European tarnished plant bug

• *L. rugulipennis* is highly polyphagous feeding on a wide range of annual and perennial weeds. Strawberry is not a highly favoured host but the pest causes damage at low population densities by feeding on the flowers and developing fruits.
• *L. rugulipennis* overwinters in the adult stage in plant debris in and around strawberry fields. It has two generations per annum in the UK, one in June and a second in August-September when the pest can be particularly numerous and when the threat to flowering strawberry crops is greatest.
• In the USA, alfalfa (*Medicago sativa*) (also known as lucerne) has been shown to be a powerful trap crop for two closely related and highly damaging capsid bug pests of strawberry, *Lygus lineolaris* and *Lygus hesperus*. Alfalfa is successfully exploited on a large scale in IPM for these pests in commercial strawberry production (Swezney et al., 2007).
• Alfalfa acts as a powerful sink for the capsids which are controlled on the trap crop by regular tractor mounted vacuuming. This approach has been very successful in the USA, and on some farms the use of insecticides for capsid bug control is no longer required.
• Practical factors have been crucial to the commercial success of alfalfa as a trap crop for capsid bugs in strawberries in the USA. It is a perennial which can be rejuvenated easily and its flowering can be controlled by timely mowing. Alfalfa seed is of very low cost and is readily available.
• Work in the UK and Sweden has shown that a number of other plant species including annual flowering herbs and cover crops are much better hosts of *L. rugulipennis* than strawberry. Large populations of *L. rugulipennis* built up on the trap crops but they then invaded the target crop as the trap crop started to senesce. The vital importance of application of a control treatment for the pest on the trap crop was highlighted in this research as well as the importance of maintaining the quality of the trap crop and preventing senescence.
• The successful use of trap cropping combined with vacuuming in USA to control the congener *Lygus* pests points to the possibility of using the same approach for *L. rugulipennis* on strawberry in the UK.
• In previous research at EMR and NRI, it was shown that one of the components of the female sex pheromone of *L. rugulipennis*, hexyl butyrate, is highly repellant to females. Capsid and related bugs are known to produce abundant ‘defensive’ volatile secretions, present in the metathoracic scent gland, that are released upon disturbance. These so-called ‘defensive’ compounds may play an important role in minimising intra-specific competition between females. Hexyl butyrate is commercially available, is comparatively inexpensive, and could be released at comparatively high rates over a long period from suitable dispensers to repell *L. rugulipennis* from the target crop. It is possible that these repellant properties could be exploited in management of the pest in commercial strawberry crops.
• The attractiveness of different plant species and their suitability for use as trap crops for *L. rugulipennis* needs to be quantified. Practical means of deploying and managing the trap crop need
to be developed. An integrated management system for *L. rugulipennis* needs to be developed which combines the use of the most promising trap crop species together with suitable control methods deployed on the trap crop. The use of hexyl butyrate as a repellent needs to be investigated to determine whether significant benefits arise if it is used in combination with a trap crop.

**Aphids**

- *C. fragaefolii* can be a serious pest on strawberries, especially those grown in the elevated temperatures under polytunnels.
- *C. fragaefolii* is a vector of three serious strawberry viruses (Shanks 1981).
- The flight period(s) of *C. fragaefolii* in UK were investigated by Dicker (1952) who showed that the aphid had a dispersal period mainly in May-June with a very small second dispersal period in the late autumn. The same pattern is shown by records from the Rothamsted Insect Survey aphid suction traps (R Harrington, pers. comm.) though numbers of aphids trapped by this method are very low in most years. Large variations in the numbers of migrants and in the numbers of aphids on different varieties were apparent.
- In commercial strawberry production in the UK, populations of aphids are controlled by spring/summer applications of insecticides which may lead to pesticide residues in the crop.
- Currently field margins are used as buffers for pesticide applications and as access areas for machinery to manoeuvre around the crop. Manipulation of field margins to provide attractive vegetation both in terms of refuges and sources of alternative food sources for predators and parasitoids of pests may increase the abundance of beneficial species in the crop area.
- In research at EMR on apple and pear, several flowering plant species were assessed for their attractiveness to a range of predatory species important as biocontrol agents (Fitzgerald & Solomon, 2004). *Centaurea cyanus*, cornflower, and *Anthemis arvensis*, corn chamomile, were found to be the most attractive species of those tested to anthocorids and *Chrysanthemum segetum*, corn marigold, was attractive to Hymenopteran parasitoids. Cornflower was also attractive to coccinellids.
- In experiments where potted pear plants infested with pear psylla, *Cacopsylla pyricola*, were placed in plots containing a mixture of the flowering plant species mentioned above or on bare soil, numbers of psyllids declined more rapidly on plants within the flowering plots, highlighting the possibilities of this habitat manipulation technique for enhancing biocontrol within the crop (Fitzgerald & Solomon, 2004). This is being assessed in a current Defra-funded project at EMR within organic apple production systems.
- Other plant species have also been shown to be attractive to beneficial species in different cropping systems (e.g. Wyss, 1995; Sengonca et al., 2002).
- Research at EMR on strawberry showed that some pest species were attracted to flowering plant species, and that pest numbers then increased in the crop (Cross, 2003). This work highlights the need for careful selection of plants for inclusion in this research.
- Synthetic herbivore-induced plant volatiles, in particular methyl salicylate, have been shown to be attractive to some beneficial species (e.g. James, 2003ab; Fitzgerald et al., 2006). In experiments at EMR, methyl salicylate was found to be attractive to *Orius* species in laboratory olfactometry experiments but not in field trapping experiments. This indicates that the release rates from the field lures were not optimal; this needs to be investigated further. Behaviour modifying chemicals were highlighted as biocontrol options warranting further investigation in HDC funded project SF 66 (Fitzgerald et al., 2005).
- In research at EMR on apple, blackcurrant and raspberry, autumn applications of insecticides have proved successful at reducing populations of a wide range of other aphid species leading to lower aphid damage in the following spring. Autumn applications of selective insecticides in strawberry, if effective, would reduce pest pressure in the following year by reducing initial populations and possibly lead to less virus transmission within the crop. Raworth et al. (1991) showed that sprays of
the systemic organophosphorus insecticide oxydemeton-methyl resulted in lower aphid numbers the following spring.

- The use of selective insecticides post harvest would also allow beneficial species present in the crop to survive, thus enabling early season biocontrol of aphids with naturally occurring or released predators and parasitoids and also enhanced biocontrol of other pests.
- In HDC funded research SF 61 at EMR (Fitzgerald, 2005) it was shown that none of the commercially available aphid parasitoids would parasitise *C. fragaefoli*. A naturally occurring species was identified from aphid mummies and identified as *Aphidius eglanteriae*. No work has been done to determine if this species could be mass produced.
- Also in SF 61 *Aphidoletes aphidimyza* was shown to have promise for biocontrol of *C. fragaefoli* but it did not prove possible to establish this species in aphid-infested strawberry plants. The species is unlikely to be able to survive the temperatures that would be encountered in early season introductions.

**Strawberry blossom weevil**

- In previous Defra funded research, EMR and NRI identified the male produced sex aggregation pheromone of strawberry blossom weevil, which attracts both males and females for the purposes of mating (Innocenzi *et al*., 2001). The pheromone has three chemical components, Grandlure I, Grandlure II and Lavandulol in a 1:4:1 ratio. A polythene sachet lure and sticky stake pheromone trap was developed in the work which is now available commercially to growers for monitoring the pest (Cross *et al*., 2006ab).
- Unfortunately, the sticky stake trap design has proved difficult to use and the attractancy of the pheromone alone inadequate. Weevils are attracted into the vicinity of the traps causing increased crop damage but many do not enter and therefore are not trapped/killed.
- Recently, there have been two further highly significant developments as a result of research work in progress currently in Norway (unpublished, but to which J Cross has acted as a scientific consultant). These are:
  1) a green funnel trap fitted with cross veins is a much more effective and practical design for use with the sex pheromone
  2) the attractancy of the sex pheromone is enormously increased if it used in conjunction with flower specific host plant volatiles
- This breakthrough provides the opportunity of developing a super trap for strawberry blossom weevil provided by combining a large surface the adult attractive, non-UV reflective white colour of strawberry flowers, key flower volatile cues used by males and females for host and oviposition site location and the known blossom weevil sex aggregation pheromone.

**Scientific Approach:**

A five-year project is proposed to reduce the incidence of pesticide residues on strawberry fruit. The first three years are primarily dedicated to developing individual components for pest and disease management and the last two years will focus on the integration of these individual components and on evaluation of such an integrated approach on a commercial scale. Reducing residues is critically important for improving food quality, minimising possible negative effects to environment/soil due to excessive use of fungicides, and maintaining consumers’ confidence in UK produce.

It is unlikely that diseases can be controlled by a single control measure. It is our belief that
reduction of pesticide usage on strawberry can only be achieved by integrating several approaches, related to disease epidemiology, agronomic practices, microbial ecology and pesticide dissipation dynamics. Figure 1 shows the research components in relation to overall disease management and strawberry production. We propose to work on three diseases: botrytis (grey mould), powdery mildew and blackspot, with emphasis on the first two. The central theme for our work on the three diseases is reduction of initial inoculum, use of prediction systems so as to apply fungicides only when necessary and use of alternative products, biocontrol agents (BCAs) and crop management close to and during the fruiting stage.

For insect pests an integrated approach using habitat manipulation, semiochemical lures, biocontrol agents together with more species specific control strategies is proposed.

For European tarnished plant bug, an IPM system for the pest will combine the use of narrow strips of a trap crop (e.g. lucerne) deployed at intervals through the crop and/or around its borders to attract the pest away from the target crop, lures containing hexyl butyrate, a defensive volatile produced by females, to repel the pest from the crop, and vacuuming the trap crop to physically remove the pest. Scientific work will involve identifying the best trap crop species and ways of managing it, examination of deployment strategies in the crop, development of suitable dispensers for hexyl butyrate, determining the range of repellancy of the lures and developing an effective deployment strategy.

For aphids, an IPM system for aphids will combine the provision of flowering herbage as sources of aphid natural enemies, semiochemical attractants to attract natural enemies into strawberry crops, biocontrol agents and end of season clean up sprays of selective insecticides. Scientific work will involve identifying ground herbage species to maximise early season populations of natural enemies, optimising semiochemical lures to attract natural enemies, development of mass rearing methods for Aphidius eglanteriae, a naturally occurring specific parasitoid of strawberry aphid and developing its use for biocontrol, and investigation of the use of late season clean up sprays of aphicides.
For Strawberry blossom weevil, a highly attractive ‘super’ trap will be developed that combines visual, host plant volatile and sex aggregation pheromone attractants and exploited for monitoring and control. Key scientific tasks are to identify flower specific host plant volatiles to synergise the activity of the sex aggregation pheromone, optimising trap design and investigating deployment strategies for effective control.