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Integrated Soft Fruit Production

editors:

Nina Trandem, Jerry.V. Cross & Christian Linder

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Biological control of grey mould in strawberry: what we know and what we need

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Abstract: This paper presents an overview of previous and ongoing research related to biological control of grey mould in strawberries (*Fragaria x ananassa*). Grey mould, caused by *Botrytis cinerea*, is a major fruit rot disease of strawberries. Symptoms can appear at all stages of fruit development if weather is conducive, and unless preventative measures are taken much of the crop will be lost. It has been known for some time that disease caused by *B. cinerea* can be suppressed by the activities of various bacteria (e.g. *Pseudomonas* spp., *Bacillus* spp.), yeasts (e.g. *Cryptococcus* spp., *Rhodotorula glutinus)* and filamentous fungi (e.g. *Trichoderma harzianum, Ulocladium atrum, Clonostachys rosea*). A number of findings suggest that, in strawberry, infection during flowering might be the stage of the disease cycle most susceptible to biological control. *B. cinerea* requires nutrients such as those found in flowers to germinate and infect plant tissues, and microorganisms which compete for nutrients have been reported to reduce grey mould in the ripe fruit. Specifically, antagonists would need to compete for nutrients from nectar and pollen during the relatively cool and humid conditions which are conducive to infection by *B. cinerea.*

A simple *in vitro* test demonstrated the importance of nutrients and temperature on interactions between *B. cinerea* and potential biocontrol agents. Potato dextrose agar (PDA) was supplemented with glucose and fructose concentrations approximating those in strawberry flowers (PDFA). These media were inoculated with a mixture of equal parts of *B. cinerea* conidia and conidia of an antagonistic fungus, i.e. *Epicoccum nigrum, Cladosporium* sp., *Trichoderma* spp., *Clonostachys* sp., or *Ulocladium* sp. Although all of the antagonists were able to inhibit *B. cinerea* to various degrees on PDA at 20°C, none were able to prevent *B. cinerea* sporulation on PDFA, and inhibition was markedly less at 15°C than at 20°C on both media. These results emphasize the importance of testing potential antagonists of flower infection on nutrient-rich substrates, at temperatures relevant to the flowering season.

Key words: fungal disease, biocontrol, plant pathogen, strawberry, *Botrytis cinerea*

A history of biological control of *Botrytis cinerea* in strawberry

Grey mould, caused by *Botrytis cinerea*, is a major fruit rot disease of strawberries. Fungicides are commonly applied throughout the flowering period to prevent development of this pathogen, which can attack leaves and flowers, as well as fruit, under conducive conditions. Concerns about fungicide residues in the fruit and in the environment, as well as the problem of fungicide resistance in the pathogen, have encouraged research into alternative methods of disease control. One such alternative is biological control using microbial antagonists.

Biological control of plant diseases is a relatively new field compared to biological control of vertebrate and invertebrate pests. To put the current research on biocontrol of *B. cinerea* in strawberry into perspective, a review of the literature in this field follows.

***Biological control of* Botrytis**

Research into biological control of *B. cinerea* started in the 1930s, when several researchers reported that *Botrytis* spp. in pure culture and on plant tissue could be inhibited by various microorganisms (literature cited in Newhook (1951)). Twenty years later, two publications described control of *B. cinerea* on lettuce by soil microorganisms (Newhook 1951, Wood 1951). Interestingly, these publications pointed out limitations to biological control of *Botrytis* that are still unsolved today: i) although simultaneous inoculation of pathogen and antagonist may prevent growth of *Botrytis* on agar and on detached leaves, inhibition on living plants requires prior inoculation of the antagonist; ii) the alternating wet and dry conditions common in the field reduce efficacy of antagonism, as *Botrytis*, unlike obligatory saprophytes, can obtain moisture and shelter as it attacks plant tissue; iii) although a considerable number of microorganisms were highly antagonistic to *Botrytis* at 25 °C, this activity decreased sharply at 15 °C and was almost absent at 5 °C.

B. cinerea *in strawberry*

The first research papers on *B. cinerea* in strawberry appeared in the 1960s, as researchers attempted to elucidate the origins of grey mould in strawberry fruits. Infection during flowering was recognized as an important source of latent infections, but the details were debated. Some researchers found that fruit rot was most likely a result of stamen infection by *B. cinerea* during flowering (Powelson 1960, Bristow et al. 1986), while others contended that the most important source of fruit infection was direct contact with infected tissues, e.g. senescent petals (Jarvis 1962, Jarvis & Borecka 1968, Bulger et al. 1987). Experimental evidence showed that *B. cinerea* conidia required exogenous nutrients for germination, and strawberry pollen promoted both conidial germination and infection of strawberries (Chou & Preece 1968, Borecka & Millikan 1973, Blakeman 1975). *B. cinerea*, like most fungi, requires free water (although perhaps only as a microscopic film) in order to germinate and infect plant tissues (Jarvis 1962).Weather during flowering thus affects the degree of infection, e.g. 24 h of wetness at 20 °C gave 100% infection of strawberry flowers (Bulger et al. 1987). High water content in plant tissues generally increases susceptibility to *B. cinerea* infection, perhaps as a result of enhanced diffusion of the pathogen's pectinases (Jarvis 1977). Frost, too, predisposes flowers to infection, even if the frost damage itself is not severe (Jarvis 1962). All the evidence thus pointed toward flowering as a critical period for infection by *B. cinerea.*

Although investigations on infection pathways and epidemiology continued, by far most of the early research on *B. cinerea* in strawberry was devoted to means of chemical control. By the time it was discovered that *B. cinerea* had developed resistance to some of the common fungicides (Bolton 1976, Maraite et al. 1980), only a few attempts had been made at biological control of grey mould in strawberry (Bhatt & Vaughan 1962, Tronsmo & Dennis 1977).

Biological control of* B. cinerea *in strawberry

After the first attempts, almost 30 years passed before additional research on biocontrol of *B. cinerea* in strawberries was reported. Systematic efforts to obtain effective biocontrol isolates were published by John Sutton and coworkers in Canada (Peng & Sutton 1991, McLean & Sutton 1992). They chose *Gliocladium roseum* (now *Clonostachys rosea*) from among the antagonistic fungi isolated from strawberry plants because it can live endophytically in leaves and suppress inoculum production by *B. cinerea* (Peng & Sutton 1991, Sutton & Peng,1993; Sutton et al. 1997). They also proposed that honeybees could be used to vector the antagonist directly to strawberry flowers (Peng et al. 1992), an idea that was later used for application of another biocontrol isolate, *Trichoderma harzianum*, to strawberries (Kovach et al. 2000). Several *Trichoderma* isolates have been commercialized as biocontrol agents for use in other

crops; however, when tested in strawberries, *Trichoderma* spp. have performed inconsistently (Tronsmo & Dennis 1977, Tronsmo 1986, Stensvand 1997 Washington et al. 1999, Hjeljord et al. 2000, Hjeljord et al. 2001, Freeman et al. 2004). Another filamentous fungus showing potential to control *B. cinerea* in strawberry is *Ulocladium atrum,* but variable results have also been reported with this isolate (Boff et al., 2001). The search for good biocontrol isolates continues, and attention has recently focussed on unicellular microorganisms. In the UK, researchers screened 559 strains of yeasts and bacteria isolated from strawberry plants; of these, 108 inhibited *B. cinerea* on agar plates, 7 were inhibitory on leaves, and two strains of bacteria, *Bacillus pumilus* and *Pseudomonas fluorescens,* were effective in a field trial (Swadling & Jeffries 1996, Swadling & Jeffries 1998). Several yeast species, e.g. *Metschnikowia fructicola, Cryptococcus albidus*, and *Rhodotorula glutinis,* have also been reported to control grey mould in strawberries, and even combinations of bacteria and yeast have been tested (Helbig 2001, Guetsky et al. 2002, Helbig 2002, Karabulut et al. 2004).

***Interactions between fungal antagonists and* B. cinerea**

Practically all studies of interactions between *B. cinerea* and fungal antagonists have been performed under controlled conditions, and little is known of antagonistic mechanisms under field conditions. Activities of microbial antagonists thought to be involved in inhibition of *B. cinerea* include competition for nutrients, space or oxygen (Blakeman 1975, Sutton 1994, Fokkema 1995, Hjeljord & Tronsmo 2003, Hjeljord & Tronsmo 2004); secretion of antifungal enzymes (Castoria et al. 2001, Saligkarias et al. 2002); antibiosis (Bélanger et al. 1995, Swadling & Jeffries 1996, Walker et al. 1996); mycoparasitism (Dubos 1987, Fokkema 1995; Elad 1996); induction of plant defenses (Elad 1996, Ippolito et al. 2000, El-Ghaouth et al. 2003); adherence to the pathogen's hyphae (Cook et al. 1997, Saligkarias et al. 2002); interference with pathogenic enzymes (Kapat et al. 1998); and reduction of virulence through viral infection (Castro et al. 2003).

Biological control in the grey mould disease cycle

Epidemiological studies point out the complexity of combating grey mould in strawberry. Biocontrol strategies are generally aimed at suppression of inoculum production, prevention of flower infection, or protection of fruit wounds. All these involve different environmental conditions. Suppression of inoculum production in the spring would require an antagonist to be competitive on or within nutrient-poor senescent tissue at low temperatures. To interfere with infection of newly-opened flowers, antagonist propagules would need to rapidly become active in the presence of readily-available nutrients, high humidity, and cool temperatures. To prevent infection of fruit or leaves in contact with infected tissues, an antagonist would have to colonize healthy plant surfaces, a relatively nutrient-poor and environmentally exposed microhabitat. To prevent infection of wounds on maturing fruit made by birds or insects would require an antagonist active on or within the fruit tissue, under the warm dry conditions which stimulate feeding activity. The final step in the grey mould disease cycle is production of sclerotia and mycelium in the autumn on plant tissues. Interference with this step in the cycle would require the antagonist to be competitive or mycoparasitic on or in plant tissues under cool wet autumn weather. During the stages of the disease cycle in which *B. cinerea* lives as a necrotrophic parasite, it is protected by host tissues from harsh environmental conditions and usually also from biological control. Thus the most probable strategy to break the infection cycle would be prevention of infection through competition for nutrients in newly-opened flowers.

Effect of nutrients on in vitro interactions between* B. cinerea *and antagonists

A simple preliminary test was devised to investigate antagonism between *B. cinerea* and common saprophytic fungi on agar plates containing sugar concentrations similar to those in strawberry flowers. One hundred strawberry flowers, fifty each of newly-opened or 2-day-old

flowers, were washed with sterile water and the sugar content of the extract was determined by gas chromatography. The flowers were found to contain equal amounts of fructose and glucose, on the average 0.5 and 1.0 mg of each sugar per new and older flower respectively. Standard potato dextrose agar containing 2% glucose (PDA) was supplemented with 2% fructose (PDFA) to approximate the sugar concentrations in flowers.

Saprophytic fungi were isolated from leaves and flowers of second-year strawberry plants (cv. Korona) on an experimental plot never treated with fungicides (Ås, Norway). Strains of *Epicoccum nigrum* (Ep1), *Cladosporium* sp. (Cl 506), *Clonostachys* sp. (Gr 313), *Ulocladium* sp. (Ulo 17), and *Trichoderma* sp. (T20), as well as a lab strain, *T. atroviride* P1, were selected for preliminary tests of antagonism toward *B. cinerea* 101, also isolated from a strawberry leaf. Conidial suspensions (2x 10^5 conidia ml^{-1}) of each antagonist were mixed with an equal amount of *B. cinerea* conidia and 10 µl drops were placed on 4 plates each of PDA and PDFA. The plates were divided into 2 batches and incubated for 7 days at 15°C or 20°C, at which time they were inspected for signs of mycelial growth and sporulation of *B. cinerea*. Control plates were inoculated with *B. cinerea* alone. The percent inhibition was estimated from the area of sporulating *B. cinerea* on the coinoculated plates compared to that on the control plates.

After 7 days on PDA at 20°C, *E. nigrum* and both *Trichoderma* strains had completely suppressed *B. cinerea* growth and sporulation on the agar surface, while the remaining antagonists suppressed *B. cinerea* sporulation around the point of coinoculation, i.e. by approximately 50% compared to the area of the *B. cinerea* control. On PDFA at 20°C, *E. nigrum* and *Trichoderma* strains suppressed *B. cinerea* sporulation by approximately 10%, while the remaining antagonists did not suppress *B. cinerea* on this medium. None of the antagonists suppressed *B. cinerea* at 15°C. These results demonstrated that the ability of all the putative antagonists to suppress *B. cinerea* growth was reduced on a nutrient-rich substrate, especially at a relatively cool temperature. The test was stringent, in that equal numbers of antagonist and pathogen conidia were employed; actual numbers of microorganisms present in susceptible flower parts varies depending on age of flower, precipitation, pollination frequency, etc. Relative concentration of fungal isolates is often cited as important to the outcome of competition between them, although the mechanisms involved are unknown.

Conclusions

Research to date has pointed out a number of environmental factors that may affect the outcome of biological control programs in strawberry crops. The most obvious is weather, particularly the amount and duration of high humidity/rain and cool temperatures. High humidity will favor survival and activity of antagonists as well as *B. cinerea*, perhaps to differing degrees, although little information on this subject is available. Environmental wetness also increases the water content of plant tissues, which may facilitate diffusion of degradative enzymes, growth of the pathogen and symptom development. Another consequence of high humidity is dilution of nectar. We have observed that *B. cinerea* conidia are unable to germinate in nectar taken from flowers grown at ≤80% RH (data not shown). However, as air humidity increases, the hygroscopic nectar absorbs water and becomes an excellent substrate for conidial germination and germ tube extension. Whether nectar dilution favors growth of *B. cinerea* or its introduced or natural competitors, e.g. the "nectar yeasts", may depend on other weather-related factors. For example, rain and low temperatures will reduce activity of the insects which introduce yeasts and other natural competitors into newly-opened flowers, while such weather will facilitate dispersal of *B. cinerea* conidia. All factors

which increase the ability of the pathogen to penetrate plant tissues will also decrease its exposure to antagonism by surface organisms.

Although low temperatures decrease the growth rates of most microorganisms, some phylloplane fungi, e.g. *B. cinerea*, *E. nigrum*, and *U. atrum*, germinate almost as quickly at 10°C as at 25°C (data not shown). Temperatures will thus affect the antagonistic ability of fungi differently, even though most have temperature optima at 20°C-25°C.

As noted above, the nutrient status of a substrate will also affect the competitive ability of fungi. In the case of strawberry flowers, the level of available nutrients will be determined by several factors. Although nectar is secreted continuously in the open flower, its availability to a microorganism will depend not only on its dilution, but also on how frequently it is collected by insects and on how many other microorganisms are competing for it. The same is true of pollen, which is also known to stimulate flower infection by *B. cinerea*.

These are but a few of the factors in the microhabitat of a flower which affect the ability of natural or introduced biocontrol agents to prevent infection by *B. cinerea*. Perhaps a search for microbial antagonists should include isolation from specific susceptible floral parts, e.g. from the nectary and anthers. Yeasts are commonly found in nectar and other sugary substrates and may represent better candidates for flower protection than filamentous fungi. We suggest that screens of potential antagonists based on careful analysis of the microhabitat to be protected may identify characteristics necessary for satisfactory levels of disease control in strawberry.

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Control of grey mould (*Botrytis cinerea*) in strawberry using fungal antagonists

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Abstract: Due to varying results when testing commercially available *Trichoderma*-products for control of grey mould (*Botrytis cinerea*) in perennial strawberry fields in Norway, we have isolated new fungal antagonists from field-grown strawberry plants to see if indigenous fungi may give better disease control. Screens of fungal isolates of different genera were carried out by inoculating detached strawberry flowers in the laboratory, and isolates were selected for field trials based on the results of the bioassay. In 2004, *Epicoccum nigrum*, *Trichoderma hamatum*, *Aureobasidium pullulans*, and *Acremonium* sp. were tested. There was a high disease pressure in the field, with 54% fruit rot in the untreated control plots. *E. nigrum* and *A. pullulans* both significantly reduced the disease incidence, to 44 and 37% rot, respectively. The other agents did not reduce disease incidence. In 2005, *Acremonium* sp and *T. hamatum* were replaced by *Clonostachys roseae* and *Ulocladium* sp., and *T. atroviride* strain P1 was also included in the trial. Heavy rainfalls during flowering caused a considerable number of flowers to die from aggressive infection by *B. cinerea*. Disease incidence in ripe fruits was low, with only 3% fruit rot in the control plots. There were no significant differences between treatments in 2005.

Key words: biocontrol, bioassay, *Botrytis cinerea*, fruit rot, indigenous fungi, strawberry

Introduction

Strawberry (*Fragaria* × *ananassa*) is an important fruit crop in Norway, and grey mould, caused by *Botrytis cinerea* Pers ex Fr., is the most important disease in this crop. Conidia of *B. cinerea* infect strawberry flowers, but symptoms usually appear at a later stage in the fruit development. Fungicides are applied regularly during flowering to protect flowers from infection. In order to reduce the use of fungicides, alternative control measures are needed. Biological control using fungal antagonists may have potential to reduce disease in strawberry (Tronsmo & Dennis 1977). Screening for potential antagonists in the laboratory has shown that many fungi are able to suppress *B. cinerea* under controlled conditions. Different species in the genus *Trichoderma* have been tested as biocontrol agents of *B. cinerea* over a long period in many countries, but field trials have shown inconsistent control (Tronsmo 1986, Stensvand 1997, Stensvand 1998). One reason for this could be that *Trichoderma* species are soil fungi, and, because they are not well-adapted to the phyllosphere, may be unable to show their antagonistic potential. In an attempt to find new biocontrol agents we have collected indigenous fungal isolates from aboveground parts of field-grown strawberry plants in Norway. This paper presents the results of two field trials with indigenous antagonists of *B. cinerea* in strawberry.

Material and methods

The fungal isolates were identified to genus, or sometimes to species by morphological characteristics. Isolates were screened for antagonistic activity against *B. cinerea* using a detached flower assay (L. Hjeljord, unpublished data). Known concentrations of spores of the potential antagonist and spores of *B. cinerea* were mixed in a water suspension. The spore suspension was applied as three 10 µl droplets in each flower. Flowers were then incubated at high humidity, at either 15 or 20°C, and examined daily for development of sepal necrosis. Isolates selected for field-testing were grown on agar medium (PDA or V8) to produce spores. Spores were scraped off the agar, and the suspensions were filtered if needed, to avoid clogging of the sprayer by fungal hyphae. The concentrations of spores in the suspensions were quantified using a haemacytometer. Concentrations of the yeasts *Aureobasidium pullulans* Au16 and *Acremonium* sp. Acr603, were adjusted to 10^8 cells per ml suspension, while the concentrations of the fungi *Trichoderma hamatum* T20, *T. atroviride* P1, and *Clonostachys rosea* (*Gliocladium roseum*) Gr313 were 10^7 conidia per ml suspension, and the concentrations of the fungi *Epicoccum nigrum* Ep1 and *Ulocladium* sp. Ulo17 were 10^5 conidia per ml suspension.

Field trials were carried out in 2004 and 2005 at The Norwegian Crop Research Institute (now Bioforsk) at Ås, Norway. The experimental field was established in August 2003 with cv. Korona in double row beds with drip irrigation. Herbicides and insecticides were applied prior to flowering, but no fungicides were applied at any time. The trial was set up as a randomised block design with three blocks, and approximately 40 plants in each plot. The field received overhead irrigation during the day (1 minute per hour from 0800 to 1900 hours) to maintain moist conditions to enhance establishment of the fungi. Application of antagonists was conducted using a knapsack sprayer (1.5 bar, 130 l/1000 m double row). In 2004, pieces of agar with sporulating *B. cinerea* Bc101 were placed in the field just before flowering to ensure flower infections. The following year, it was assumed that the build-up of natural inoculum from the previous year was sufficient for disease development. In 2004, five sprays with the antagonists were carried out over a two-week period, and in 2005 eight sprays were carried out over a three-week period. In both years the field was harvested three times per week for a total of 12 and 9 times in 2004 and 2005, respectively. Berries infected through contact with diseased fruits were defined as healthy in these experiments, since the investigation was aimed at preventing infection during flowering. Flowers and fruits showing symptoms of grey mould were harvested as soon as they were detected, and healthy berries were harvested when ripe. At all harvest dates in 2004 and at two harvest dates in 2005, samples of healthy berries from each treatment were incubated at room temperature to test for latent infections of *B. cinerea*.

Statistical analysis was performed using the analysis of variance (GLM-procedure of Minitab 14.20), and treatments were compared using Tukey's test ($P = 0.05$). Data on accumulated daily precipitation and mean daily temperatures for both seasons were obtained from a local weather station at Ås.

Results and discussion

The bioassay showed that the fungal isolates had different ability to prevent *B. cinerea* from causing sepal necrosis. In flowers inoculated with *B. cinerea* only, symptoms started to appear after three days, and after 5-6 days sepal necrosis had developed from 100% of the inoculum droplets. Co-inoculation of potential antagonists in spore suspension with *B. cinerea* reduced the incidence of sepal necrosis in the flowers. *E. nigrum* gave the best result of the fungi in

the bioassay and was able to reduce sepal necrosis to 0 – 35% compared to the control inoculated with *B. cinerea* only. The corresponding reduction in sepal necrosis for *T. atroviride* P1 was 63-83%. All the fungi selected for field trials were able to reduce sepal necrosis incidence, but the efficacy was largely dependent on the relative spore concentrations of the pathogen and the antagonist. In general, the antagonists were more effective when concentrations were higher than the concentrations of the pathogen, compared to if they were inoculated at the same concentration.

In 2004, four isolates were tested in the field; *T. hamatum*, *E. nigrum*, *A. pullulans*, and *Acremonium* sp. There was a very high disease pressure in the field, with 54% fruit rot in the control plots (Figure 1). Treatment with *E. nigrum* and *A.* pullulans both significantly reduced fruit rot incidence compared to the control plots (by 19 and 31 percent, respectively, P = 0.004). *T. hamatum* and *Acremonium* sp. did not reduce fruit rot incidence compared to the control, but treatment with *Acremonium* sp. resulted in significantly less fruit rot than treatment with *T. hamatum*. In 2004, the flowering season was characterised by dry weather, and only 11.2 mm accumulated precipitation was recorded during the period of spraying. The harvest season had much more rainfall, with 122.8 mm accumulated precipitation. The wet weather coincided with berry ripening and probably triggered development of stem-end rot of *B. cinerea*, which we assume originated from flower infections. As water films are necessary for spore germination of *B. cinerea* (Jarvis 1962), flower infection was probably favoured by overhead irrigation, but it is also possible that dewfall during night may have provided sufficient free water for the fungus to infect the flowers. Symptoms developed in incubated berries 2-3 days after picking, but there were no significant differences in post harvest rot between the treatments. The combination of high disease pressure and wet weather during harvest favoured disease development, and therefore the results of this field trial indicate that indigenous antagonists have a potential as biocontrol agents of *B. cinerea* in strawberry.

In 2005, we tested the same isolates of *E. nigrum* and *A. pullulans* as in 2004, but we replaced *T. hamatum* and *Acremonium* sp. with *C. rosea* and *Ulocladium* sp. In addition, we included *T. atroviride* P1, which has been tested in several earlier field trials (data not shown). Weather conditions in the 2005 season were opposite from the previous year. During flowering 85.4 mm rain was recorded, while the harvest season was dry with 32.2 mm rain. A considerable number of flowers were killed by *B. cinerea* during flowering because of the wet weather combined with a high disease pressure. Approximately half of the potential yield was lost in the field due to flower blight prior to fruit development, and there were no significant differences between treatments (Figure 2). The yield loss due to Botrytis fruit rot was very low, with only 3% in the control plots. There were no significant differences between the treatments, neither at harvest nor in the post harvest tests.

The results of these trials indicate that the precipitation pattern during the season had a great influence on when symptoms of *B. cinerea* flower infections appeared. Heavy rains during flowering promoted disease in earlier stages of development. Sprays with antagonists in the wet flowering season did not reduce flower blight compared to the control plots. The reason for this could be that the antagonists were unable to inhibit aggressive infection of the pathogen promoted by the climatic conditions, or that rainfall after spraying washed away the antagonist spores. The preliminary conclusions from this work are that indigenous antagonists show potential in reducing fruit rot caused by latent flower infection with *B. cinerea*, but aggressive flower infections may be more difficult to prevent. Future work includes testing of antagonists under different controlled humidity conditions, and studies of mechanisms of antagonistic activity.

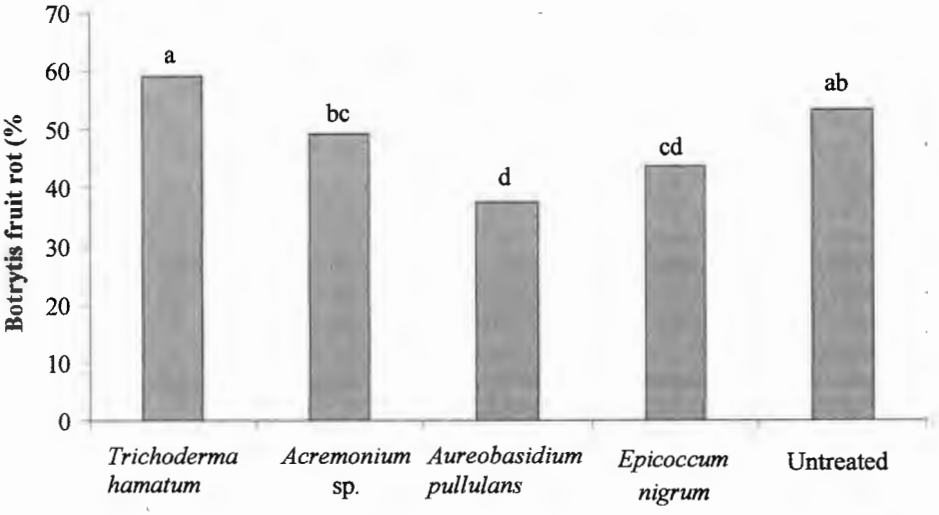

Figure 1. Control of grey mould (*Botrytis* cinerea) in strawberry cv. Korona, by spraying spore suspensions of indigenous, antagonistic fungi during flowering, Ås, Norway, 2004. Mean of three replicates, significant differences indicated by different letters on top of the bars (Tukey's test, P = 0.05).

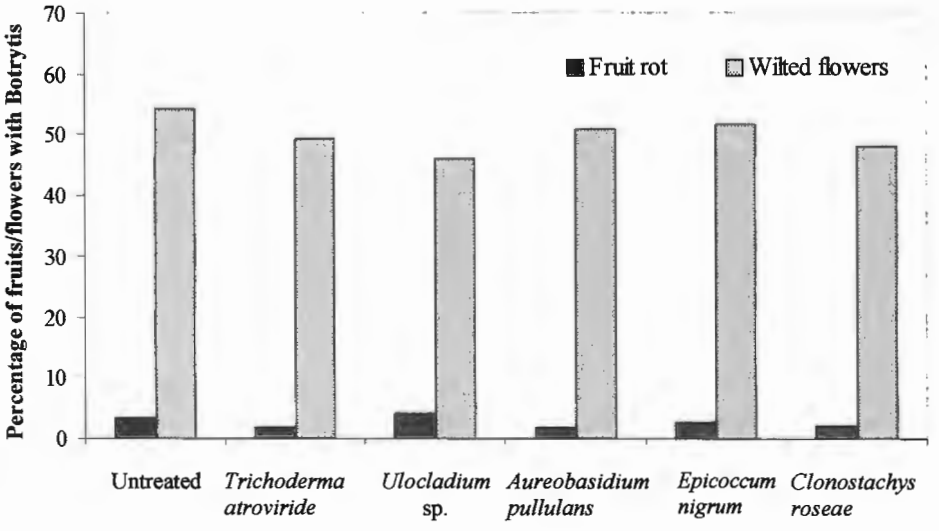

Figure 2. Control of grey mould (*Botrytis cinerea*) in strawberry cv. Korona, by spraying spore suspensions of indigenous, antagonistic fungi during flowering, Ås, Norway 2005. Mean of three replicates.

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Effectiveness of Switch 62.5 WG and Signum 33 WG for the control of strawberry grey mould (*Botrytis cinerea* Pers.) and reduction of two-spotted spider mite (*Tetranychus urticae* Koch) populations

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Abstract: Two new fungicides Switch 62.5 WG and Signum 33 WG were tested for control of strawberry diseases. Their efficacy against grey mould was about 90 and 80%, respectively. Both products effectively reduced the intensity of powdery mildew and leaf spot. During the studies, a side effect of the tested fungicides was observed: a reduction of the two-spotted spider mite population. The spider mite control achieved with Switch 62.5 WG was similar to that of the standard fungicide, Euparen Multi 50 WG, which showed the highest efficacy. The efficacy of Signum 33 WG was satisfactory for mite control but its activity was shorter because the population of mites rebuilt earlier than after application of Euparen Multi 50 WG.

Key words: *Botrytis cinerea,* broad spectrum of activity, fungicides, grey mould, *Tetranychus urticae,* two-spotted spider mite, strawberry

Introduction

In strawberry plantations in Poland, fungal diseases such as grey mould (*Botrytis cinerea*), leaf spot (*Mycosphaerella fragariae*), powdery mildew (*Sphaerotheca macularis*) and some pests, especially two-spotted spider mite (*Tetranychus urticae*), are species of very high importance (Łabanowska and Meszka 2003) causing significant losses. Grey mould affects fruits whereas leaf spot, powdery mildew and spider mites damage the leaf tissue. All of them should be controlled every year. The number of fungicide treatments depends on weather conditions (Jarvis 1964, Bulger et al. 1987), cultivar (Baruzzi et al. 1997, Łabanowska and Bielenin 2002) and age of plantation. Because the Integrated Production system has become very popular in recent years, growers use fungicides with broad spectrum of activity. Our earlier experiments (Łabanowska and Bielenin 1997, Meszka et al. 2000) showed that some chemical products used for control of grey mould showed some positive effect on reduction of other diseases and pests: leaf spot, powdery mildew and two-spotted spider mite populations. In recent years, new generation fungicides were tested against strawberry fungal diseases (Stensvand, 1998). Newly developed compounds, Switch 62.5 WG (cyprodinil and fludioxonil) and Signum 33 WG (piraclostrobin and boskalid), were registered in Poland in 2005.

The aim of this work was to evaluate the effect of Switch 62, 5 WG and Signum 33 WG, used in various programs of grey mould control, on leaf spot and powdery mildew severity and on development and reduction of two-spotted spider mite populations on strawberry.

Material and methods

The experiments were done in 2003-2005 on 2 or 3 strawberry plantations located in Dąbrowice (A) and Miedniewice (B), near Skierniewice, in Central Poland and Witowice (C) near Puławy (south - east Poland). Experiment on grey mould and leaf spot were performed in 2 – 3 year old plantations of cv. Senga Sengana and on powdery mildew on cv. Elsanta. Each experiment was designed in strip blocks where a plot of approximately 60m^2 represented one treatment (combination), which was subdivided into four blocks. The following fungicidcs were evaluated: Sumilex 500 SC (procymidone), Euparen Multi 50 WG (tolylfluanid), Switch 62.5 WG (cyprodinil+fludioxonil) and Signum 33 WG (piraclostrobin+boscalid). The applications were made with motor knapsack sprayer "Solo" at a volume rate of 600 l/ha. First treatment was made at the beginning of bloom and the next at the intervals of 5-7 days. Dates of treatment were as follows:

2003: 16, 20, 26 May and 2 June
2004: 14, 18, 28 May and 4 June
2005: 21, 27, 30 May and 1 June

The occurrence of grey mould was evaluated on fresh fruits (400 randomly selected fruits per treatment) collected during harvest and on fruits stored for 48 hrs at room temperature (about 20°C in 2003-2004 and 4.5 °C in 2005). The severity of leaf spot and powdery mildew were assessed in field on 400 randomly selected leaves in each combination (100 leaves per replication). The severity of symptoms was evaluated using the following scale: 0-no symptoms, 5- more than 80% surface of leaf covered by *S. macularis* (powdery mildew) and more than 50% by *M. fragariae* (leaf spot).

In the same experiments the influence of some fungicides on two-spotted spider mite populations was evaluated. The two-spotted spider mite population density was estimated 3 times during the growing season. The active stages of mites and eggs were counted separately, using the Henderson & McBurnie (1943) brushing technique. The samples of 30 fully developed leaves taken from each of 4 replications of each treatment (total 120 leaves). The results were analysed statistically on data transformed according to the logarithmic function $y = \log (x+1)$, where x was the number of mites per 30 leaves. Cumulative Index of Infestation (CII) were calculated according to the formula of Wratten *et al.* (1979) and then recalculated into percentage assuming CII for the untreated control = 100. All results are presented in tables 1-3 and figures 1,2.

The significance of differences between means was evaluated using Duncan's multiple range t-test at P=0.05.

Results and discussion

Tested fungicides provided a satisfactory control of grey mould (about 90 and 80% efficacy), even in weather conditions favourable for development of the pathogen (Table 1 and 2). Efficacy of Switch 62.5 WG and Signum 33 WG was higher or similar to that obtained with standard fungicides like tolyfluanid (Euparen Multi 50 WG) and procymidone (Sumilex 500 SC). A good effect of Switch 62.5 WG against tomato grey mould was observed by Tort et al. (2005). In our earlier study it was observed that Switch 62.5 WG applied only according to predicted infection periods (two treatments) was very effective. It should be pointed out that in 2002 the effectiveness of such a programme applied on the very susceptible cultivar Senga Sengana was similar to the conventional one, in which Switch 62.5 WG was used four times

during the blossom period (preventive treatments) (Meszka and Bielenin 2004). In these tests, we observed that both fungicides also showed good protection against very serious diseases such as powdery mildew (*Sphaerotheca macularis*) and leaf spot (*Mycosphaerella fragariae*). Their effectiveness in reduction of these diseases was about 70% (Fig.1 and 2).This finding is particularly important for strawberries grown for the fresh market.

During 2003-2005, in the experimental strawberry plantations, the density of two-spotted spider mite (*Tetranychus urticae* Koch) population was not so high. The number of mites on the plants sprayed with fungicide Euparen Multi WG, Switch 62.5 WG and Signum 33 WG was much lower than on untreated control plants. The best reduction of mites was noted after using Euparen Multi 50 WG. The results with tolyfluanid as Euparen M 50 WG correspond with those obtained earlier (Łabanowska and Meszka 2003). A good reduction in mite populations also occurred with Switch 62.5 WG. The efficacy of Signum 33 WG in reducing the number of two-spotted spider mite was satisfactory, but the population of mites rebuilt earlier than after application of Euparen M 50 WG (Table 4 A-C).

Table 1. Effectiveness of Switch 62.5 WG for control of grey mould on strawberry cv. Senga Sengana, in two plantations (A and B), 2003 – 2005.

Fungicide and rate per 1 ha	Percent of affected fruits during harvest and after 48 hrs storage at 20°C or 4.5 °C temperature*				
	2003		2004	2005	
	A	B	A	A	B
Untreated control	19.8 c	16.4 c	45.2 c	59.8 c	39.0 c
Switch 62.5 WG 1.0 kg *(cyprodinil + fludioxonil)*	0.06 a	2.9 a	5.2 ab	8.2 a	5.6 a
Euparen Multi 50 WG 5.0 kg *(tolylfluanid)*	3.0 b	6.3 b	11.2 b	26.9 b	24.8 b
Sumilex 500 SC 1.5 l *(procymidone)*	2.3 b	2.2 a	3.6 a	X**	X

* Statistical analysis was made separately for each year and each plantation. Means in columns followed by the same letter do not differ at 5% level of significance (Duncan's multiple range t-test)
** X = not tested

Table 2. Effectiveness of Signum 33 WG for control of grey mould on strawberry cv. Senga Sengana, at two plantations (A and B), 2004 – 2005.

Fungicide and rate per 1 ha	Percent of affected fruits during harvest and after 48 hrs storage at 20°C or 4.5 °C temperature*			
	2004			2005
	A	B	C	A
Untreated control	77.2 b	45.2 c	16.1 b	54.5 c
Signum 33 WG 1.8 kg *(piraclostrobin+boskalid))*	7.3 a	12.5 b	0.5 a	7.0 a
Switch 62.5 WG 1.0 kg *(cyprodinil + fludioxonil)*	X**	5.2 ab	X	5.5 a
Euparen Multi 50 WG 5.0 kg *(tolylfluanid)*	9.7 a	11.2 b	1.7 a	26.9 b
Sumilex 500 SC 1.5 l *(procymidone)*	X	3.6 a	3.9 a	X

Footnotes, see Table 1

Table 3. Average number of two-spotted spider mite (*T. urticae*) per leaf of strawberry ('Senga Sengana')

A) 2003

Fungicide and rate per ha		Number of motile mites			CII
		4 June	30 June	28 July	(control=100)
Untreated control		1.9 d*	1.3 b	0.05 ab	100.0 b
Sumilex 500 SC	1.5 l	0.7 c	1.3 b	0.04 ab	67.8 ab
Euparen Multi 50 WG	5 kg	0.4 b	0.6 a	0.02 a	32.2 a
Switch 62.5 WG	1 kg	0.2 a	1.3 b	0.02 a	58.2 ab

Footnote, see Table 1

B) 2004

Fungicide and rate per ha		Number of motile mites			CII
		22 June	12 July	20 July	(control=100)
Untreated control		16.2 c*	3.1 b	3.9 d	100.,0 c
Sumilex 500 SC	1.5 l	32.7 d	5.7 b	0.7 c	198.1 d
Switch 62.5 WG *preventively*	1.0 kg	2.8 b	0.1 a	0.2 b	16.4 a
Switch 62.5 WG *according to infection*	1.0 kg	7.0 b	4.5 b	3.9 d	68.4 b
Euparen Multi 50 WG	5.0 kg	0.4 a	0.2 a	0.1 a	3.6 a
Signum 33 WG	1.8 kg	4.9 b	4.9 b	3.7 d	63.6 b

Footnote, see Table 1

C) 2005

Fungicide and rate per ha		Number of motile mites			CII
		29 June	6 July	15 July	(control=100)
Untreated control		1.8 a*	0.4 a	0.1 a	100.0 a
Switch 62.5 WG	1,0 kg	1.0 a	0.2 a	0.1 a	49.6 a
Euparen Multi 50 WG	5.0 kg	0.7 a	0.1 a	0.0 a	27.1 a

Footnote, see Table 1

Conclusions

1. The new fungicides Switch 62.5 WG and Signum 33 WG used at rates of 1.0 kg and 1.8 kg per 1 ha, respectively, gave very good control of the strawberry grey mould.
2. Both fungicides used in a programme for grey mould control also reduced infection by leaf spot and powdery mildew.
3. When used to control grey mould these fungicides also showed some acaricidal effect and reduced two-spotted spider mite (*Tetranychus urticae* Koch) populations. The best results were with Euparen Multi 50 WG and Switch 62.5 WG.

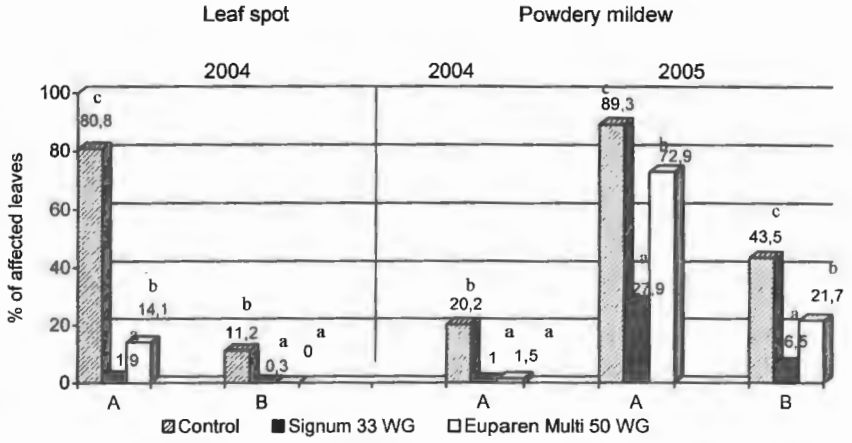

Figure 1. Effectiveness of Signum 33 WG for control of leaf spot (cv. Senga Sengana) and powdery mildew (cv. Elsanta) in 2004-2005.

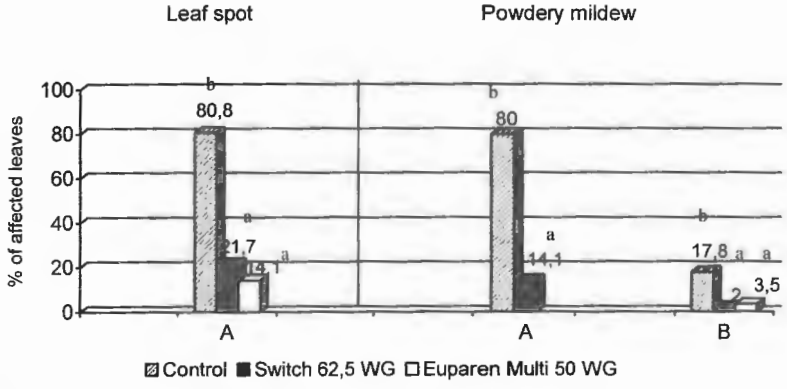

Fig 2. Effectiveness of Switch 62.5 WG for control of leaf spot (cv. Senga Sengana) and powdery mildew (cv. Elsanta) in 2004.

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Biological control of thrips and aphids in tunnel-grown strawberry in UK

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Abstract: Experiments were carried out in the laboratory, glasshouse and field to determine the effectiveness of several biocontrol agents against the strawberry aphid *Chaetosiphon fragaefolii* and thrips, in particular western flower thrips *Frankliniella occidentalis*, on strawberry. In preliminary glasshouse experiments, *Orius majusculus*, *O. laevigatus* and the nematode *Steinernema feltiae* (Nemasys F) significantly reduced thrips numbers but did not eliminate them. In laboratory experiments none of the commercially available parasitoids, *Aphelinus abdominalis*, *Aphidius ervi*, *Aphidius colemani* and *Aphidius matricariae*, parasitised *C. fragaefolii*. The predatory midge *Aphidoletes aphidimyza* was effective in potted plant glasshouse experiments but did not establish after mass releases in the field on tunnel grown strawberries.

Key words: aphids, *Chaetosiphon fragaefolii, Frankliniella occidentalis,* parasitoids, predators, strawberry, thrips

Introduction

Several species of thrips occur on strawberry in the UK (Easterbrook 1991, Fitzgerald 2004), and feeding by thrips on strawberry fruits can cause serious crop damage in late season strawberries. Western flower thrips (*Frankliniella occidentalis*), a non native species in UK, poses a significant threat as it is resistant to a number of available insecticides (e.g. Ludwig & Oetting 2001), and is favoured by the elevated temperatures found under polytunnels.

The strawberry aphid, *Chaetosiphon fragaefolii*, can be a serious pest on strawberries, especially those grown under polytunnels. The aphid is a virus-vector, and the honeydew they produce causes the fruits to become sticky and unmarketable.

Many species of naturally occurring predators and parasitoids are found in strawberry plantations (Easterbrook 1998) but numbers often lag behind pest population growth so they are unreliable as biocontrol agents, especially early in the season. Several species of commercially available predators have been assessed for their potential to control or reduce populations of thrips or various aphid species on glasshouse grown crops (e.g. Van Schelt 1994, Sterk & Meesters 1997, Ebssa *et al.* 2001, Jacobson *et al.* 2001, Sanchez & Laasa 2002, Bennison *et al.* 2005) but this has not been done on tunnel grown strawberries. For control of thrips these biocontrol agents have included *Orius* spp., *Neoseiulus (Amblyseius) cucumeris*, and entomopathogenic nematodes. For control of various aphid species they include the predator *Aphidoletes aphidimyza* and the parasitoids *Aphelinus abdominalis* and *Aphidius* spp. The potential use of parasitoids to control aphids in strawberry was highlighted in a review of biocontrol agents in strawberry (Cross *et al.* 2001). However, parasitoids have specific host preferences; the commercially available species have not been tested to determine if they will parasitise the strawberry aphid. The aim of these experiments was to assess the effectiveness of a range of commercially available biocontrol agents against pests on tunnel grown strawberries.

Methods

Biocontrol of western flower thrips (WFT)

Potted strawberry plants were infested with WFT by shaking thrips off plants from laboratory cultures. Six batches of 16 plants were placed into separate glasshouse compartments (3 x 2 m), held at 20 °C. Five plants were sampled at random to determine initial thrips numbers; all leaves, flower and fruiting clusters were examined under a stereomicroscope.

Treatments were releases of the predatory insects *Orius majusculus* (Syngenta Bioline) and *O. laevigatus* (Biological Crop Protection), the predatory mite *Neoseiulus cucumeris* (BCP), the insect parasitic nematode *Steinernema feltiae* (as Nemasys F; Becker Underwood) and an untreated control. Owing to the dispersive capacities of the predator species and the certainty of cross contamination if treatments were set up in the same glasshouse, it was necessary to co-locate the replicates of each treatment within a single glasshouse. However, one treatment, *O. laevigatus*, was replicated in two glasshouses in order to obtain an indication of the degree of glasshouse to glasshouse variability. Release rates for the two *Orius* species were 1 per plant (equivalent to 10 per m^2; 10 females and 6 males were released per compartment), and for *N. cucumeris* 10 per plant (equivalent to 100 per m^2; c 70% of those released were adult females). Nematodes were applied using a hand sprayer at the equivalent of the curative application rate, where one pack (50 million infective juveniles) treats 50 m^2. The glasshouse compartment was humidified before the nematode applications by drenching the walls and floor with water. Applications were made late afternoon (18.00 hours), and the compartment remained humid for around one hour after application. Two releases of predatory mites and insects were made, one on 15 June and the second on 14 July. Four weekly applications of nematodes were made.

Two weeks after the initial treatment six plants were taken at random from each compartment and numbers of thrips present on all plant parts recorded under a stereomicroscope. Six weeks after initial treatment the remaining ten plants were sampled from each compartment. Counts were compared with an analysis of variance.

***Effect of parasitoids on* Chaetosiphon fragaefolii:**

Immature and adult *C. fragaefolii* were placed onto detached leaves and kept in ventilated containers. Hop leaves infested with *Phorodon humuli*, the damson hop aphid, were placed in separate boxes; *P. humuli* was used as a control species as it was known from previous work at EMR that several of the parasitoids used in these experiments would parasitise this aphid species.

Three species of parasitoids are widely available from biocontrol suppliers in UK and were obtained from Syngenta Bioline. These were *Aphidius ervi*, *Aphidius colemani* and *Aphelinus abdominalis*. *Aphidius matricariae*, although a UK native species, is not available from suppliers in UK. This species was obtained from Canada by Syngenta Bioline for this research. Adult parasitoids were sexed and three pairs released into each container. The containers were then put into a constant temperature (CT) room at 20°C, with 16 hours light per day. After eleven days a sample of 10 aphids from each replicate leaf was dissected under a stereomicroscope to determine if any of them contained developing parasitoid larvae. The remaining aphids were kept for a further week to check for visible signs of developing parasitoids (aphid mummies).

***Effect of* Aphidoletes aphidimyza *on* C. fragaefolii**

Consumption rates: *Aphidoletes aphidimyza* pupae were obtained from BCP and Syngenta Bioline. Eggs laid by emerged adults were used in the experiments. *A. aphidimyza* eggs and second and third instar nymphs of *C. fragaefolii* were transferred onto strawberry leaf discs held in small plastic pots on a layer of moist sand, so that each leaf disc supported a single *A.*

aphidimyza egg and 10 *C. fragaefolii* nymphs. The plastic pots were placed in a large unventilated plastic box and held at 20 °C. The leaf discs were checked daily, and the date of egg hatch and the daily numbers of aphids eaten by the *A. aphidimyza* larvae were recorded. The remains of consumed and dead aphids were removed and replaced with fresh aphids. As the consumption rate of the *A. aphidimyza* larvae increased the number of aphids presented on the leaf disc was increased to ensure there was always a surplus present.

Fecundity of *A. aphidimyza*: The fecundity of *A. aphidimyza* females was determined on detached strawberry leaves with *C. fragaefolii* kept in containers as described above. A male and female adult *A. aphidimyza* less than 24 hours old were placed in each container and numbers of eggs laid after four days were recorded.

Effectiveness of *A. aphidimyza* as a control agent for *C. fragaefolii* - potted plant experiment: Strawberry plants were standardised by reducing them to 5 expanded leaves and 1 or 2 folded leaves from a single crown. The numbers of aphids on each leaf were recorded under a stereomicroscope. The treatments consisted of 0, 2 and 10 *A. aphidimyza* larvae per plant. Two-day-old larvae were transferred to the plants and held at 20 °C with 16 hours light. Six days after releasing the *A. aphidimyza* larvae the aphid numbers were again assessed. Counts were log transformed and compared by an analysis of variance.

Effectiveness of *A. aphidimyza* as a control agent for *C. fragaefolii* - field experiment: The experiment was done on a purpose-planted plot at EMR. Each treatment plot consisted of 20 plants, planted in a double row raised bed through polythene. The plants were 0.5 m apart in the row and 0.4 m apart between rows. There was a 5 m gap between plots in each bed and a 3 m gap between beds. The planting was covered by plastic portable multi-span (Spanish) tunnels. On 16 July mass releases of *A. aphidimyza* were made in four plots. Pupae were placed on moist paper inside ventilated plastic boxes, and when adults had emerged (50-100 per box) the boxes were taken to the field and the midges released into the canopy in the centre of each plot. A sample of 50 trifoliate leaves was taken from each release plot on 19 July to determine if *A. aphidimyza* had established.

Results

Biocontrol of western flower thrips

The mean number of thrips (adults plus nymphs) per plant pre-treatment was 26. Mean numbers two and six weeks after initial release are presented in Table 1.

Two weeks after release, there was a significant 51% reduction of total thrips only in the *O. majusculus* treatment ($P<0.05$). Six weeks after the initial release, numbers of thrips were significantly lower than in the control in all except the *N. cucumeris* treatment. Reductions were 56% for the Nemasys F treatment ($P<0.01$), 52% for the *O. majusculus* treatment ($P<0.05$) and 42% for the *O. laevigatus* treatment ($P<0.05$).

***Effect of parasitoids on* C. fragaefolii**

Only one parasitoid larva was found in 454 *C. fragaefolii* dissected; this was an *Aphidius ervi*. In contrast, in *P. humuli*, 168 out of a total of 288 aphids dissected contained parasitoid larvae. Percentage parasitism in *P. humuli* was 45% by *Aphidius ervi*, 86% by *A. colemani*, 89% by *A. matricariae* and 14% by *Aphelinus abdominalis*. When the hop leaves were inspected a week after the dissections, many mummies were present. No mummies were found in any of the boxes containing *C. fragaefolii*

Table 1. Mean numbers of thrips per plant after release of biocontrol agents.

Treatment	Mean number of thrips (all stages)	
	2 weeks after initial release	6 weeks after initial release
Untreated[1]	34.7	92.4
Orius majusculus[1]	17.0	44.8
Orius laevigatus[2]	22.8	53.3
Neoseiulus (Amblyseius) cucumeris[1]	42.2	73.2
Nematode[1]	25.8	40.6
SED[1]	8.21; 31 df	18.21; 55 df
SED[2]	7.11; 31 df	15.77; 55 df

Each treatment [1] was replicated 6 and 10 fold 2 and 6 weeks after release respectively
Each treatment [2] was replicated 12 and 20 fold 2 and 6 weeks after release respectively

***Effect of* Aphidoletes aphidimyza *on* C. fragaefolii**

Consumption rates: The mean number of *C. fragaefolii* consumed by *A. aphidimyza* larvae during their development was 45. Larval development took a mean of 6 days. Larvae that had fed on *C. fragaefolii* pupated successfully.

Fecundity of A. aphidimyza: The mean number of eggs laid on *C. fragaefolii* infested leaves by *A. aphidimyza* over a four day period was 46. Adults lived for a maximum of 6 days.

Effectiveness of *A. aphidimyza* as a control agent for *C. fragaefolii* - potted plant experiment: The effectiveness of releases of *A. aphidimyza* is shown in Table 2. Both release rates of *A. aphidimyza* reduced numbers of *C. fragaefolii* on the strawberry plants, and the higher release rate was more effective than the lower rate.

Table 2. Effect of *Aphidoletes aphidimyza* larvae on numbers of *Chaetosiphon fragaefolii* on strawberry plants. Log transformed means are in parentheses.

A. aphidimyza release rate	Aphid numbers post treatment
0	45.8 (1.494)
2	36.2 (1.404)
20	28.1 (1.258)
LSD P<0.01 df 148	0.134

Effectiveness of *A. aphidimyza* as a control agent for *C. fragaefolii* – field experiment: In the leaf sample taken on 20 July, after the mass release of *A. aphidimyza* adults on 16 July, a total of 3 *A. aphidimyza* larvae were found in 200 leaves. Anthocorid and syrphid eggs were seen on these leaves. Most leaves had less than 10 aphids.

Discussion

Orius majusculus, *O. laevigatus* and the nematode preparation Nemasys F significantly reduced thrips numbers but did not eliminate them. Biocontrol agents generally take some time to effect control of pests. Higher release rates would give better control, but the cost may

become uneconomic. Other possibilities are to release low numbers of *Orius* early in the season, or when the plants are flowering (*Orius* species will feed on pollen and may thus become established before pest problems increase), or to make multiple releases of lower numbers of predators (technical information from Biocontrol companies), *Orius* adults are mobile insects and may leave the crop if pollen or prey is not available.

In detached leaf and potted plant experiments *A. aphidimyza* showed promise as a biocontrol agent for *C. fragaefolii*. Adults laid eggs among *C. fragaefolii* colonies, and larvae were able to develop successfully on this aphid species. When larvae were released onto potted strawberry plants they significantly reduced aphid numbers. However, from the field experiments there was no evidence that *A. aphidimyza* had successfully established in this planting. Difficulty in establishing this predator has also been seen in other experiments (Richard GreatRex, Syngenta Bioline, personal communication). Although the larvae consumed reasonable numbers of aphids in the laboratory experiments, this species has a relatively short larval developmental stage and spends longer in the pupal stage (14 days at 23 °C). This means that multiple releases of the predator may be required to give effective control of aphids. *A. aphidimyza* pupate in the soil, which may be a problem where strawberries are grown through polythene mulch. Different release techniques may have more success in establishing this predator in the field; Syngenta Bioline now produces this product in 'bubble packs' which are claimed to be more effective for pupal emergence and enable the adults to mate before emerging from the pack (R. GreatRex, Syngenta Bioline, personal communication).

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Use of *Neoseiulus* (*Amblyseius*) *cucumeris* to control *Phytonemus pallidus* under field conditions

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Abstract: In this study the predatory mite *Neoseiulus cucumeris* was used to control the strawberry mite *Phytonemus pallidus*. The experiment was conducted under field conditions in two cultivars of strawberry, 'Honeoye' and 'Cavendish'. The strawberry plants were also treated with pyrethrum and pyrethrum + fleece to control the strawberry blossom weevil, *Anthonomus rubi* (result not discussed here). The results show significant differences in number of *P. pallidus* between the cultivars, i.e. more strawberry mites on 'Cavendish'. The results also show that the population of *P. pallidus* was affected both by the release of *N. cucumeris* and the pyrethrum and pyrethrum + fleece treatments. In plots with *N. cucumeris* the number of *P. pallidus* increased significantly when treated with pyrethrum and fleece. In plots without *N. cucumeris,* there were no significant differences between the two pyrethrum treatments and the untreated control but there were significantly more *P. pallidus* when the plants were treated with pyrethrum + fleece compared with pyrethrum.

Key words: *Neoseiulus cucumeris*, *Phytonemus pallidus*, pyrethrum, strawberry, integrated control

Introduction

The strawberry mite, *Phytonemus pallidus,* is an important pest in strawberries in Sweden. In organic production the possibilities to control this mite are very limited. One way is to use the predatory mite *Neoseiulus cucumeris* (Acari: Phytoseiidae) (Easterbrook et al. 2001). However, a strawberry field is rarely attacked by a single pest and combinations of treatments are often necessary. Another important pest in Sweden is the strawberry blossom weevil, *Anthonomus rubi*. Previous work at Rånna Experimental Station (unpublished data) has shown a decreased number of buds clipped by the weevil when using a combination of fleece and pyrethrum.

Material and methods

Cultivation practices

The strawberries were planted in August 2001 on black plastic mulch. The plants were irrigated with overhead sprinklers and fertilized with vinasse (a by-product from the production of yeast) and dried pelleted poultry manure. Straw was applied as winter mulch and also at harvest to keep the berries clean. Cultivars used were 'Honeoye' and 'Cavendish'.

Treatments

One year after planting, furled leaves infested with *P. pallidus* were evenly spread in the field to ensure a population of strawberry mites. In half of the plots 40 *N. cucumeris* per plant was released in the beginning of June and July in 2003 and 2004. To avoid movement of the predatory mite between the plots, the inter-row spaces between the main plots were kept weed-free.

In this experiment the plants were also treated with pyrethrum and the combination of fleece and pyrethrum to control the strawberry blossom weevil, *Anthonomus rubi* (results not

shown here). The strawberry plants were covered with fleece and sprayed twice between the end of April and the beginning of June, i.e. before the release of *N. cucumeris*.

***Number of* P. pallidus**

The number of *P. pallidus* was counted on ten furled leaves per plot using a stereo-microscope. The samples were taken every other week from the beginning of June to the end of September and at the end of October in 2003, and every other week from the beginning of June to the beginning of August in 2004.

Yield

The strawberries were harvested on 12 occasions in 2003 and on 19 occasions in 2004. The marketable yield was classified by visual inspection.

Statistical analyses

The experiment had a split plot design with four replicates. The main plot consisted of the treatment against strawberry mites and the subplots were the cultivars and treatments against strawberry blossom weevils. Each subplot consisted of 30 strawberry plants. The data were analyzed by Analysis of Variance (GLM) using the Statistical Analysis System 6.12 (SAS Institute, Cary, NC, USA, 1989-1996). Significance level was 5 %.

Results and discussion

Differences between cultivars

The results showed large significant differences in number of *P. pallidus* in the two varieties. The mean number of mites per leaf was 3 and 16 on 'Honeoye' and 'Cavendish' respectively in 2003, and 2 and 17 in 2004.

Pyrethrum and fleece affected populations in 'Cavendish'

The results also showed that the effect of *N. cucumeris* on *P. pallidus* was affected by the pyrethrum and pyrethrum + fleece treatments.

In plots with *N. cucumeris* the number of *P. pallidus* was higher when treated with pyrethrum and the combination of pyrethrum + fleece compared with control. The population was kept quite constant throughout the season. The fact that *N. cucumeris* was spread after the sprays with pyrethrum indicates that pyrethrum affects other natural enemies in the field. Pyrethrum is known to have a short-term effect (Katsuda 1999) but is a non-selective pesticide and can harm beneficials (Simmonds et al. 2002).

In plots without *N. cucumeris* the pattern was somewhat different. Here the number of *P. pallidus* followed the order; pyrethrum + fleece > control > pyrethrum and the population reached a maximum in the end of August and in September. Figure 1 shows the mean number of *P. pallidus* in 2003.

Population size affected yield the following year

The size of the population of *P. pallidus* did not affect the yield measured the same year. Although the differences were not significant, p=0.149, large populations in the autumn seemed to affect the yield in 'Cavendish' negatively the following year. The yield decreased by 35% in plots with high numbers of mite compared with plots with low numbers. This is also reported by Stenseth & Nordby (1976). They showed that more than 35 strawberry mites in average per leaflet in the post-harvest period, reduced the number of flowers the following year.

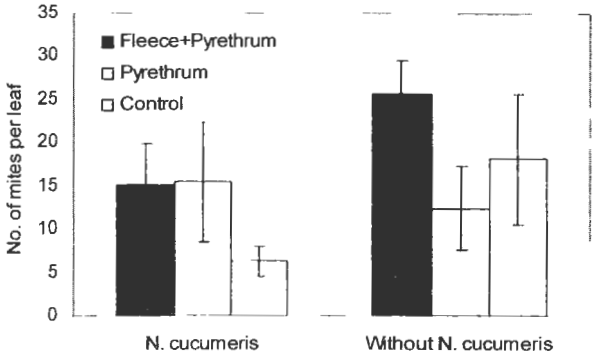

Figure 1. Mean number of strawberry mites, *P. pallidus* on young folded strawberry leaves in 2003. Y-error bars show standard error of the mean.

Acknowledgements

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Control of the black vine weevil *Otiorhynchus sulcatus* Fabricius (Coleoptera: Curculionidae) on strawberry crops in Ireland

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Abstract: The black vine weevil, *Otiorhynchus sulcatus* Fabricius (Coleoptera: Curculionidae) is the most important pest of Irish strawberries. The withdrawal of the effective organochlorines in the early 1990s has left growers with no alternative effective control. In the last decades entomopathogenic nematodes (EPN) in the families of Heterorhabditidae and Steinernematidae are being used as biological control agents for this pest. Trials were carried out in grow bags outdoors, indoors in a polythene house and outdoors on black polythene mulch. Trials in grow bags resulted in black vine weevil larval mortality of 93.4% with *Heterorhabditis megidis* and 51.3% with *H. downesi.* In the indoor trials all EPN treatments were effective, with the triple application being the most successful (resulting in 96.8 % mortality of black vine weevil larvae). In the outdoor trials on black polythene mulch similar results were achieved, with the triple application being the most effective (resulting in a mortality of 91.4%).

Keywords: black vine weevil, entomopathogenic nematodes, biological control.

Introduction

The black vine weevil (BVW) *Otiorhynchus sulcatus* is a serious pest of soft fruit, ornamental and nursery plants (Fitters et al. 2001, vanTol 2004). Damage to the host is mainly caused by the larvae feeding on the roots (Moorhouse 1992, Smith 1932}, which may result in reduced vigour and subsequent death of the plant (Garth 1978, La Mondia 2005).

The two genera of entomopathogenic nematodes (EPNs) that are currently receiving most attention as biocontrol agents are *Heterorhabditis* and *Steinernema.* EPNs have been successfully used to control black vine weevil larvae in potted plants and glasshouse crops (Simons 1981, Kakouli-Duarte 1997, Fitters *et al.* 2001, Lola-Luz *et al.* 2005). In this work the effectiveness of the entomopathogenic nematodes for the control of black vine weevil larvae, on strawberries grown under protection and in the field in Ireland was investigated.

Materials and methods

Indoors

Strawberries, cv Elsanta were grown indoors, they were planted in 5 liter pots. The pots contained medium grade peat based compost. The distance between the rows was 80 cm and between the pots 40 cm. Field collected BVW adults were released at a rate of 5 BVW/pot on the 8th of June 2003 and 40 melanised BVW eggs/pot on the 24th of July 2003. The experiment was set up in a randomised complete block design with 4 treatments: Single (September) EPN application, Double (September, October) EPN applications, Triple (September, October, March) EPN applications, and Water only control (3 water applications). Each replication consisted of 5 pots (20 plants). There were in total 10

replications per treatment. Each planting was assessed in spring, usually 13-14 days after the last EPN application. The numbers of BVW/pot were recorded, and all plants of the planting were destroyed in this process.

Field

Strawberries of cv Elsanta were planted on raised beds. The strawberry beds were 20 cm high and each bed accommodated 2 rows of plants. The distance between the rows and between plants within the row was 30 cm. A plot (replication) consisted of 20 and each plot was separated from other plots in the same bed by four buffer plants. The experimental design was a complete randomised block design with 10 replications of each of 5 treatments. The five treatments were: Single (September) EPN application, Single (October) EPN application, Double (September, October) EPN application, Triple (September, October and April) EPN application and Water only control (4 water applications). All 10 blocks were assessed in May 2004. The EPN tested in both indoor and field experiments were *Heterorhabditis megidis* (UK211).

Grow bags

The nematodes tested were the UK isolate of *Heterorhabditis megidis* (UK211) and the Irish isolate *H. downesi* (K122). The nematodes were cultured in late instar larvae of the greater wax moth (*Galleria mellonella* (L.) from The Mealworm Co., Sheffield, UK) at 20 °C. The infective juveniles were recovered in White traps (White 1927). The emerging infective juveniles were washed three times by sedimentation in tap water and then stored in shallow water in plastic food containers (Roundstone Catering, UK) at a concentration of 2000 nematodes/ml. The nematodes were stored at 7 °C for four weeks or until used in the experiments.

Strawberry plants were grown in commercial growing bags (40 l) containing medium grade, peat based, compost (Bord na Mona). Eleven holes were opened in each bag and one Elsanta plant was placed on each opening. The plants were cropped for their first year in a polythene tunnel. In their second cropping year, raised beds were constructed outdoors and covered with Mypex®, to suppress weed growth. The bags were then placed on top of the raised beds. A complete randomised block experimental design was used. The compost around each plant was inoculated with 25,000 nematodes in 55 ml of water. The untreated control plants received only 55 ml of water. Nematodes were applied on: 17/5/01, 2/10/01 and 14/5/02. Ten days after each nematode application nine blocks were randomly chosen and the compost in each grow bag was thoroughly examined for larvae of *O. sulcatus*. Numbers of all alive and dead larvae were recorded. The assessed bags were then discarded. Soil temperatures were recorded by temperature sensors connected to loggers (Unidata, Starlogger, UK); other parameters were obtained from MetEirean, the Irish meteorological service.

Results

Indoors

The Friedman's test showed that there were significant differences among the treatments (df=3 and P=0.000). When the control treatment was omitted, significant differences remained and this was attributable to the high effectiveness of the triple application (Figure 1).

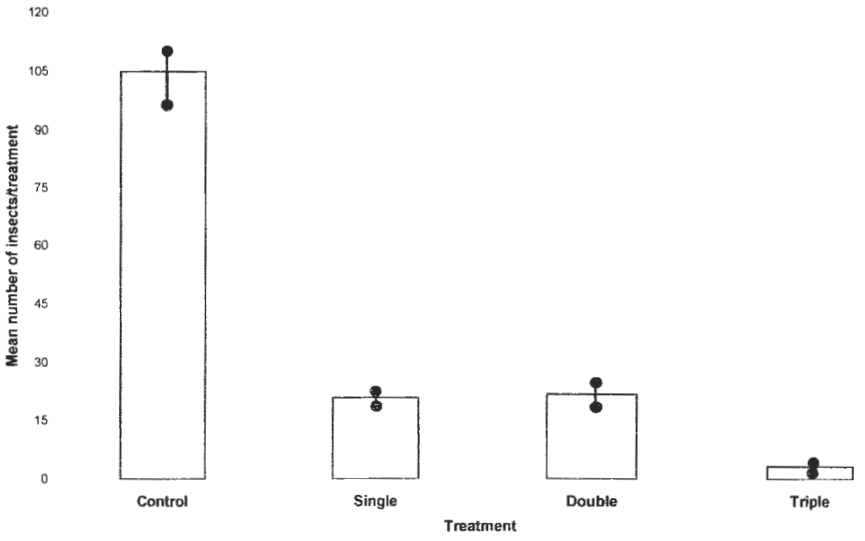

Figure 1. Mean number of live BVW recovered in each treatment in the indoor trial. Overall treatments differed at P=0.000 (Friedman's test).

Field

Friedman's test comparing all treatments was highly significant (df =4, P=0.000). This was attributable to high counts of live insects in the control treatment and to the low numbers in the triple application (Figure 2). There was no statistical evidence of differences between the remaining treatments. The highest number of dead insects was recovered from the plots that had received nematode treatment in April.

Grow bags

The mean number of live insects of *O. sulcatus* recorded is presented in Figure 3. Except in May 2001, when some pupae and 4 adult weevils were found, on the other two assessment dates (October 2001 and May 2002) all *O. sulcatus* were in their larval stage. Overall in all three application dates, the two isolates reduced the number of live black vine weevil larvae and pupae relative to the control (Figure 3). On all three assessment dates, 100% of the larvae found in the control growing bags were alive. In the first application date (May 2001) there were significant differences among treatments in the number of live insects recovered (Friedman's, df=2, P<0.005). In addition to that there were significant differences between the two nematode species, *H. megidis* and *H. downesi*, which resulted in 93.4% mortality for the former and 51.3% for the latter. The trend of reduced number of live black vine weevil larvae, recovered from growing bags that had received treatment with entomopathogenic nematodes was confirmed in the second (October 2001) application, when *H. megidis* induced 78.9% and *H. downesi* 77.6% mortality. Similar results were obtained in the third (May 2002) application date when *H. megidis* induced 93.7% and *H. downesi* 88.1% mortality. However, there were no differences detected between the two nematode species.

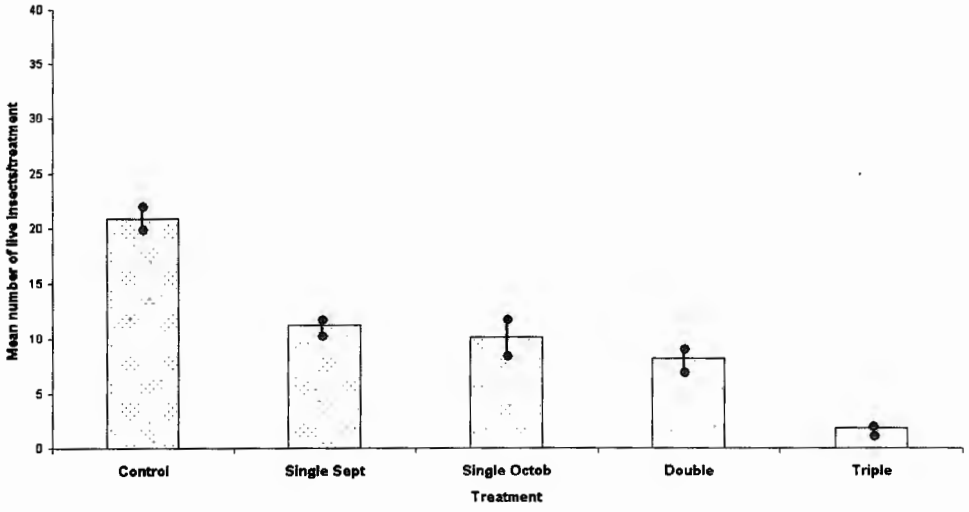

Figure 2. Mean number (±SEM) of live insects following a single, double and triple applications of *H. megidis* in the field (2003-2004) (Friedman's test, df=4, P=0.000). When the triple treatment was omitted significance disappeared (P = 0.313).

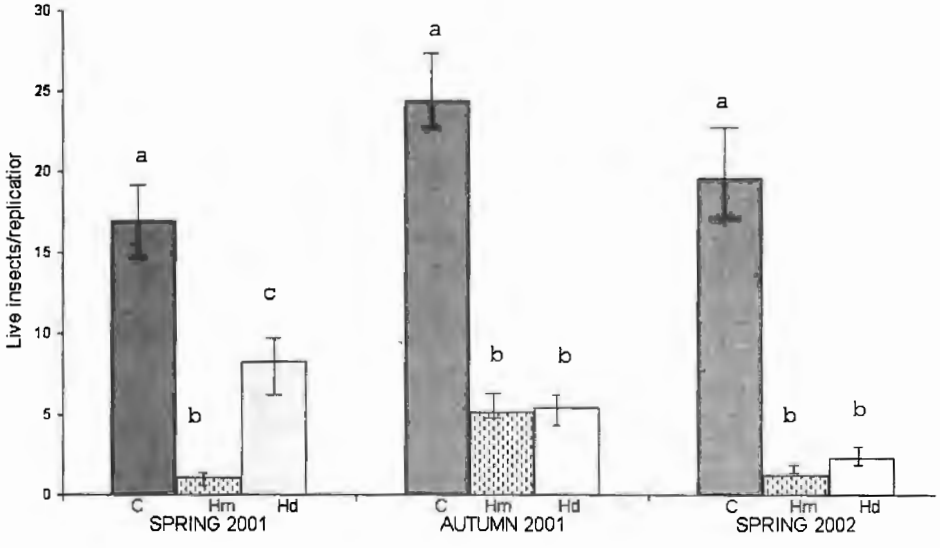

Figure 3. Mean (SEM) number of live insects, following one, two and three applications of EPNs. Different letters above bars indicate statistical differences (Friedmans, df=2, P<0.01). (C: Control, Hm: *H. megidis*, Hd: *H. downesi*)

Discussion

Several studies on the control of BVW with entomopathogenic nematodes under protection and in the field have indicated the potential of these biocontrol agents against vine weevil (Kakouli-Duarte 1997, Gill 2001, Fitters 2001, Lola-Luz 2005).

All our trials indicated effective control of BVW, with the triple EPN application being more effective that the single and double treatments. Of particular interest were the results from the EPN application in the field, due to the low temperatures that occur in Ireland during the time of application. Results were very promising and indicated that effective control of BVW is possible in outdoor crops in Ireland, with the multiple application being more effective.

The highest level of control was achieved with the triple application of nematodes, which was also significantly different from all other treatments. This is in agreement with all the other experiments that were carried out for the control of the weevil.

Acknowledgements

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Using entomopathogenic nematodes against *Otiorhynchus* in field grown strawberries – does it work?

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Abstract: The use of entomopathogenic nematodes (EPN) against the vine weevil *Otiorhynchus sulcatus* was studied under field conditions. A number of field trials were conducted using two commercial products of EPN, Nemasys H (*Heterorhabditis megidis*) and Nemasys L (*Steinernema kraussei*). The results from these trials indicate that low temperature is still a limiting factor for the successful use of EPN against *O. sulcatus* in Northern Europe. Furthermore it was observed that application methods of EPN in field grown strawberries require improvement.

Key words: biological control, entomopathogenic nematodes, *Heterorhabditis megidis*, *Otiorhynchus sulcatus*, root weevils, *Steinernema kraussei* , strawberry

Introduction

In Norway strawberries are produced mainly outdoors, using direct drills or black polythene mulch. The production area is currently about 1725 ha, with an estimated annual value of 345 mill. NOK (43 mill. Euros). Most strawberry production occurs in the southern part of the country, and it is in the southernmost and western part that root weevils are major problem. There are several species of root weevils that are associated with strawberries in Norway, but it has been shown that the most damaging species is the vine weevil *Otiorhynchus sulcatus* (Stenseth, 1979; Hesjedal, 1982; Moorehouse et al., 1992). Post harvest application of the pesticide azinphosmethyl against the adult weevils is the most common control method today. Azinphosmethyl will no longer be available from 2006 and there are currently few alternative pesticides on the market that are effective against vine weevil adults.

Entomopathogenic nematodes (EPN) are an option for controlling the soil dwelling larvae. In Norway there are three products on the market comprising two species (*Heterorhabditis megidis* and *Steinernema kraussei*). Nemasys H and Nematop (*H. megidis*) are not recommended at temperatures below 12°C. Nemasys L (*S. kraussei*) is a cold active product that is recommended for use in late autumn at temperatures below 12°C. In 2004 - 2005 a number of field trials were conducted to examine the efficacy of the commercial cold active product Nemasys L in field grown strawberries. Nemasys H was also used in the experiments as a standard. Some of the results from these trials are presented here.

Material and methods

A total of 5 field trials were conducted, four in the Southern part of the country and one in the North Western part. Each trial was set up as randomised blocks along strawberry rows, with four plots per treatment comprising 16 plants. EPN treatments were applied manually as a drench per plant in a 100 ml volume with doses of 30000, 25000 or 15000 per plant. Treatments were conducted in early spring or late autumn to target the over wintering larvae. The spring treatments were assessed after about one month and the autumn treatments after 6-

7 months (overwinter). At each assessment 8 plants per plot were dug up and the number of live *O. sulcatus* larvae or pupae per plant were counted. Comparisons were made between untreated control plants and EPN treated plants using the mean number of larvae per plant (in blocks).

Results and discussion

In the first spring trial, temperatures were unusually mild for the time of year and soil temperatures did not dropped below 12°C. In this trial Nemasys H and Nemasys L worked quite well at the highest dose per plant (30000/plant) applied two times with a week's interval. The mean number of larvae per plant was reduced by 89% and 77% for the Nemasys H and Nemasys L treatments respectively. For all the following trials, two spring trials and two autumn trials, soil temperatures were always below 12°C on average, ranging from 6°C to 9°C in early spring and late autumn to below 0°C in winter. In these trials Nemasys H did not work well at all (always less than 30%) and confirms that this nematode product does not work at low temperatures. Nemasys L unfortunately did not perform as well as expected. In the Nemasys L treatments the number of larvae was reduced to, at best, 50% and 64% at a dose of 25000 and 30000 per plant. In strawberry fields infested with root weevils, the damage threshold is quite low (2-5 larvae per plant). Plants are usually attacked by at least two or more larvae, which is sufficient to reduce yields or kill the plant. Therefore a 50% to 60% effect of a nematode treatment is not acceptable.

Nemasys L (*Steinernema kraussei*) is reported to be effective at temperatures down to at least 5°C according to trials conducted in England (Willmott et al., 2002). Similar effectiveness could not be demonstrated for the field trials conducted in Norway. One of the reasons for this could be that the field trial in England was conducted in a more controlled manner, whereby strawberry plants in pots containing a known number of weevil eggs were buried in the field outdoors and treated individually with nematodes. In the Norwegian trials naturally infested strawberry fields were used and nematodes were applied in a similar manner to the English trial but on to plants that were growing under black plastic mulch.

It appears that the practical application or use of EPNs in open fields might result in rather inconsistent or poor results. Application of EPNs in such a way that they are able to reach and infect the pest target in the soil and root system, has to be improved.

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Asymmetrasca (*Empoasca*) *decedens* Paoli (Homoptera, Typhlocybinae): a new pest of cultivated red raspberry in Trentino, Italy

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Abstract: In 2003, the green leafhopper *Asymmetrasca decedens* Paoli was recorded for the first time on red raspberry in Trentino, alpine region in the north-east of Italy. The damage of this new pest was spectacular, especially on the autumnal fruiting variety Polka, recently introduced in Trentino. In 2004 and 2005, we carried out field surveys in order to collect information about the biology and behaviour of the pest, its damage on raspberry, how to monitor populations, and possibilities of control. Preliminary results suggest that, on cultivated raspberry, the pest may complete 2-3 generations from the end of May to the end of October. The second generation is the most damaging on autumnal fruiting varieties. During the season, part of the *A. decedens* population may also develop on various other hosts, from which adults may continuously migrate to raspberry. The combined use of yellow sticky traps and visual leaf inspection represents a valuable method to monitor the population development of the pest on the crop and to time treatments correctly. Amongst the chemicals permitted on raspberry in Italy and tested with a single application on the peak of the first nymphal generation, Trebon (ethofenprox) gave the best results. Smart EW (malathion) had no effect. Very promising results were obtained with Actara (thiamethoxam) and Calypso (thiacloprid), but none of these are registered on soft fruits in Italy.

Key words: *Asymmetrasca* (*Empoasca*) *decedens*, biology, chemical control, green leafhopper, monitoring, raspberry

Introduction

At the end of the summer 2003, a new pest of cultivated red raspberry was detected for the first time in Trentino, alpine region in Italy; the green leafhopper *Asymmetrasca* (*Empoasca*) *decedens* Paoli.

The first inspection in the plantation where the pest was found, revealed an high population (800 adults/yellow sticky trap and 1.3 nymphs/complete leaf) and a spectacular damage, especially on the autumnal fruiting cv. Polka. Worried about this new pest, the technical advisers of the local growers requested information about the biology and behaviour of the leafhopper, the damage on raspberry, and possible methods for monitoring and control. Here we present the preliminary results of our investigations carried out in 2004 and 2005.

Material and methods

Population development, biology and monitoring

Observations were carried out in a plantation of about 5000 m^2, situated in Mocheni's Valley, at an altitude of about 650 m.a.s.l. The plantation (plantation A) consisted of 80% 'Heritage' and 20% 'Polka', both autumnal fruiting varieties. The surveys were carried out in Polka. Other information was collected in 2005 from another field (plantation B) of about 600 m^2

with Heritage, situated at 1100 m.a.s.l in the same valley. The flight of *A. decedens* adults was investigated using yellow sticky traps (10 x 25 cm, Kollant® s.p.a, Padova, Italy), placed vertically in trees and shrubs along the border (Plantation A only; 7 traps), and in the crop canopy (7 traps in A, and 5 in B). The nymphal population on raspberry plants was investigated by means of visual field inspections of complete leaves collected every 7-15 days. In 2005, leaves were collected from basal, median and apical portions of the canes (10-15 complete leaves/portion/block).

Beating shrubs and wild plants bordering the fields and sweep-netting weeds were used to get additional information about the alternative hosts of the pest. We also did a literature survey.

Chemical control

In 2005, a trial was carried out in both the plantations with the aim to evaluate the effectiveness of 3 insecticides, malathion, ethofenprox and thiamethoxam, against *A. decedens*. These sprays were applied at the peak of the first generation nymphs, with the aim to evaluate their effectiveness on the following generations as well. The trial was organised in blocks, without replications (Table 1). Chemicals were applied by means of a Galaxy sprayer gun mounted on a barrow equipped with a motor-driven pump. The operating pressure was about 15 bar. Unfortunately, in plantation A, the grower sprayed with ethofenprox on the whole plantation the 24th of June.

Table 1. Experimental set-up and data of chemical treatments in the plantations.

Plantation	Block and treatment	Surface	Rate	Application volume (l/ha)	Application date
A	*1 - Control*	200 m^2	unsprayed		
	2 - ethofenprox (Trebon)	200 m^2	100 ml/hl	2500 l/ha	17 June 2005 (*)
	3 - malathion (Smart EW)	200 m^2	150 ml/hl	2500 l/ha	17 June 2005
	4 - thiametoxam (Actara)	200 m^2	400 g/ha	2500 l/ha	17 June 2005
B	*1 - Control*	300 m^2	unsprayed		
	2 - thiametoxam (Actara)	100 m^2	400 g/ha	2500 l/ha	04 July 2005
	3 - ethofenprox (Trebon)	100 m^2	100 ml/hl	2500 l/ha	04 July 2005

(*) the grower sprayed with ethofenprox on all the blocks the 24th of June

Results and discussion

Geographical distribution and host plants of the pest

A. decedens is particularly concentrated in many countries of the Mediterranean area, such as Spain, France, Italy, Serbia-Montenegro, Greece, Libya, Israel, Egypt, Cyprus, etc.

It is a highly polyphageous species, which can feed on several cultivated and wild arboreous plants (such as peach, almond, plum, apricot, cherry, apple, grapevine, citrus, birch, *Salix* spp., *Ulmus* spp., *Populus alba*, etc.), on many herbaceous crops (strawberry, beans, beet, egg-plants, tomatoes, potatoes, sweet pepper, lucerne, etc.) and on weeds (*Amaranthus retroflexus*, *Chenopodium album*, *Solanum nigrum*, *Rubus* spp., etc).

In Italy, the pest first appeared in the south (Calabria, Campania) and then in northern regions (Toscana, Veneto, Emilia Romagna, Piemonte and Trentino). It may have been introduced in Trentino some years ago, by means of infested plant material. Rather mild winters and very hot and dry summers in the last years may have supported the establishment of the pest and its local spreading and shifting from primary hosts to new ones (such as the cultivated red raspberry), and the development of large populations. The poor growth of raspberry canes during summer 2003 (due to high temperatures and water stress), and the high susceptibility of the cv. Polka, may have contributed to amplify the damage.

Description of the damage on raspberry

Adults and nymphs are phloem feeders, sucking sap from the leaves by inserting their stylet into the petioles and the main veins on the underside. They feed especially on young, tender tissues on the top of canes, laterals shoots and flower clusters.

This feeding activity induces alterations in plant tissues. The veins of infested leaves show chlorotic spots and become wavy. On the most infested leaves, the veins are necrotic. The lymphatic vessels may be occluded and leaves become more or less curled and twisted. The most damaged leaves may turn yellow, starting from the margins, and then dry up and die.

When the infestation is particularly severe (as in 2003), many new small leaves are produced on the top of canes, shoots and clusters. The internodes are very shortened, so these leaves are agglomerated.

Both the nymphs and especially the adults can also damage the ripe fruits; silvery discolourations and depressions appear on the drupelets, which then shrivel and do not ripe.

Biology, population development and damage

Data and field observations collected (summarised in figures 1-3), and information from literature (Viggiani et al., 1992; Jacas et al., 1997), help us to describe the probable life cycle of the pest on raspberry in Trentino:

Adults of green leafhoppers that occur on raspberry in Trentino belong to a complex of species, of which *A . decedens* and *Empoasca vitis* Göthe are the most common. The adults of *A. decedens* spend the winter on evergreen weeds and plants outside the crops. They start to leave these refuges very early the next spring, during the first sunny and warm days of March (Fig.1 – traps in borders). In both years studied, most of the first adults in plantation A were caught on border traps situated in front of a small wood of conifers. Therefore, it is possible that this was the preferred overwintering site in this place. The first fertilised adult females make short and rapid flights on new grass and weeds around and inside the plantation; they probably feed and start to lay their eggs on these hosts, until new raspberry canes emerge from the soil (normally from the beginning of April). In the first half of May, when canes are about 30-50 cm long, the flight of over-wintered adults peaks (Fig.1). Many adults then arrive on the new raspberry canes and lay their eggs inside tender apical leaves. At this time of the season, *A. decedens* dominates the catches of adult green leafhoppes on sticky traps.

The first young nymphs appear about 20 days later on basal leaves (< 40 cm; Fig.1 and 2); they feed for about 5 weeks, passing through 5 instars before reaching the adult stage. The first nymphal generation develops in June and July on the underside of leaves on basal and median portions of the canes, between 30 and 100 cm of height (Fig.1 and 2). These infested leaves are more or less distorted and curled, but usually this damage is of modest importance and does not affect the regular growth of the canes.

A second flight period starts at the end of June/beginning of July and finishes at the end of August/beginning of September, with a peak around the end of July (Fig.1,3). This flight produces a second generation of nymphs, partially overlapped with the first one, that develops from about the middle of July, till the middle of September, with the peak at the end of

August (Fig.1-3). As observed in 2003, this is the most harmful generation for autumnal fruiting varieties of raspberry. The feeding activity of adults and nymphs, which in this period is particularly concentrated on apical leaves rather than on basal and median ones (Fig.2), may prevent the regular formation of flower clusters and seriously injury the foliage on the apex and the ripening fruits.

A third more or less complete nymphal generation can occur in September/October, especially in the case of particularly favourable climatic conditions (as we had in 2003).

The dynamic of the pest population recorded in 2005 in the plantation B, seems to be a little bit delayed compared to that recorded in the plantation A (Fig.1 and 3); this may be due to a cooler climate in the first site. Beatings and sweep-nettings confirm that part of *A. decedens* population develops on various wild hosts around the raspberry crops.

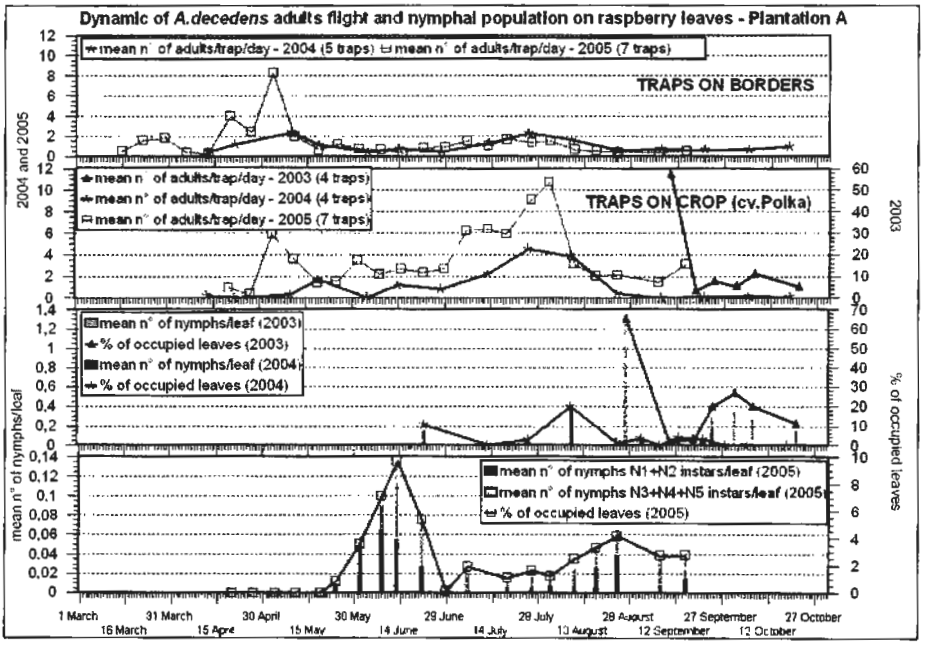

Figure 1. Dynamic of *A. decedens* adults flight and nymphal population on raspberry leaves in Plantation A.

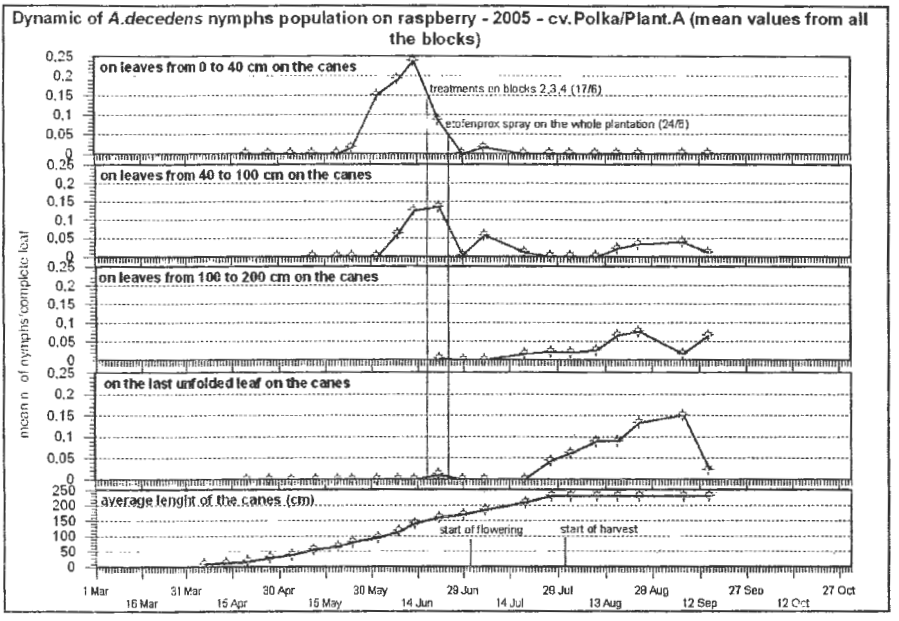

Figure 2. Dynamics of *A. decedens* population on raspberry leaves from different portion of the canes in Plantation A.

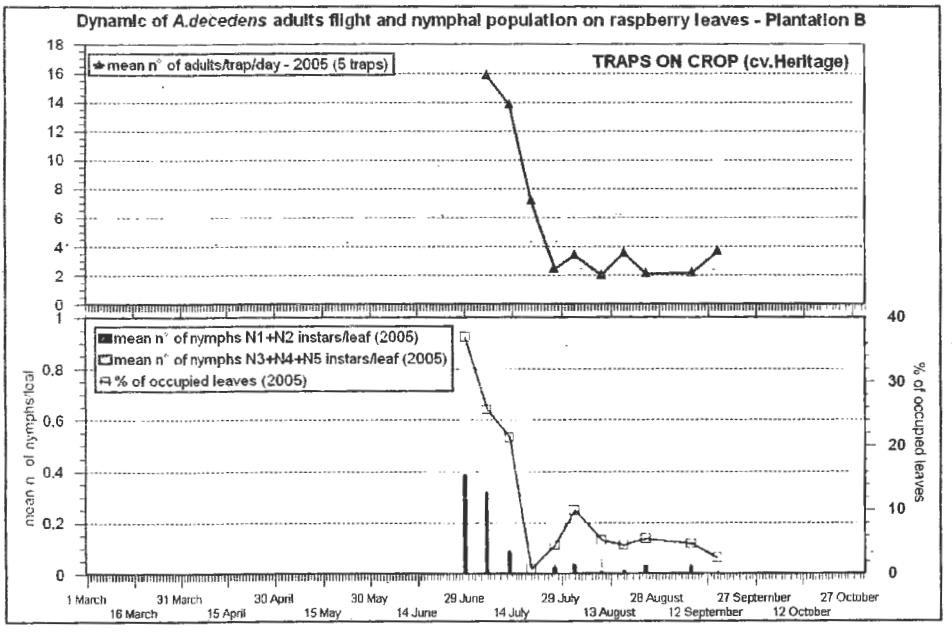

Figure 3. Dynamics of *A. decedens* flight and nymphal population on raspberry leaves, Plantation B.

Monitoring of the pest

Yellow sticky traps placed in the raspberry crop as soon as new canes emerge, may help the growers to continuously check the risk of infestation for the crop and to time the visual inspections of the leaves correctly. The leaf inspection is the only method to ascertain the presence of the pest on the canes, to assess the numerical size of the populations, the control thresholds and the effectiveness of treatments. A good linear relationship between the percentage of complete leaves occupied by nymphs and their density (R^2=0.986 and 0.959 respectively for plantation A and B), suggests that a practical sampling method based on the percentage of leaves with nymphs may be developed for the growers. Visual leaf inspection at 7-10 days intervals is recommended in June/July, during the first generation (inspecting the leaves between 30 and 100 cm on the cane), and during the second generation of nymphs, in August, just after the peak of adult catches on sticky traps (inspecting the leaves above 100 cm and on the top of the canes).

Effectiveness of insecticides

Amongst the insecticides we tested, ethofenprox (Trebon) and thiamethoxam (Actara) gave the best results (Table 2).

Table 2. Effectiveness of 3 insecticides tested against *A. decedens* in 2005.

Plantation A	Mean n° of nymphs/leaf							
Block	**pre-treatment** (13 June)	**4** days after treatment	*Application date (grower treatm.)*	**5** days after grower treatment	**12** days after grower treatment	**25** days after grower treatment	**33** days after grower treatment	**54** days after grower treatment
1 - *control*	0,18	0,14	24 June	0	0,01	0	0	0,05
2 - ethofenprox	0,19	0		0	*not inspected*			
3 - malathion	0,15	0,2		0,01	*not inspected*			
4 - thiametoxam	0,13	0		0	0	0	0	0,02

Plantation B	Mean n° of nymphs/leaf							
Block	**pre-treatment** (29 June)	**2** days after treatment	**9** days after treatment	**16** days after treatment	**23** days after treatment	**29** days after treatment	**44** days after treatment	**51** days after treatment
1 - *control*	0,95	0,97	0,7	0,01	0,08	0,17	0,08	0,09
2 - thiametoxam	0,8	0,04	0	0	0	0	0	0
3 - ethofenprox	0,38	0	0	0	0	0,03	0	0,02

They have a fast and strong control action on the pest, and a long persistence (up to 50 days for thiamethoxam in plantation B). These features may guarantee that 1 or 2 well timed applications on the peak of the first nymphal generation, may be sufficient to avoid a high population during the second generation of the green leafhopper.

Insecticides must be applied on the first nymphal generation, since the second one occurs during the flowering and ripening of the fruits on autumnal fruiting varieties, when insecticides are forbidden.

Unfortunately, thiamethoxam is not registered on soft fruits in Italy, and multiple sprays with ethofenprox often cause severe outbreaks of two spotted spider mite (*Tetranychus*

urticae). Malathion (Smart EW), another insecticide permitted on soft fruits in Italy, does not seem to have any effect on *A. decedens*. Very promising results were obtained in other trials with thiacloprid (Calypso) and acetamiprid (Epik), but none of them are registered on soft fruits in Italy.

Acknowledgements

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Introduction of certification in propagation of planting material of soft fruits in the Republic of Serbia

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Abstract: Long-term practice of establishment of new plantings with plants originating either from commercial plantings or mother plantings of unreliable phytosanitary status has brought about numerous problems in raspberry growing in the Republic of Serbia. Yield reduction, poor fruit quality and shortened exploitation life of plantings are evidenced as major problems. In view of the fact that trueness-to-type of a cultivar as well as healthy planting material are major preconditions for successful fruit production, the work on certification of the planting material of the *Rubus* genus has been conducted at the Fruit and Grape Research Centre in Čačak since 2004.

Key words: raspberry, planting material, certification scheme

Introduction

Raspberry belongs in a group of economically most relevant fruit varieties in the Republic of Serbia (Petrović and Leposavić 2004). Total raspberry growing area in our country is about 15,000 ha, whereas the production ranges from 75,000 to 93,000 t. The majority of plantings were established by the plantings originating from commercial plantings, which actually accounts for low yields and poor fruit quality, coupled with short exploitation life of raspberry plantings (Mišić et al. 2004). By the Law on seeds and planting material (Official Gazzette of the Republic of Serbia, num. 54/1993) utilization of thus propagated plants has been prohibited. New Law (Official Gazzette of the Republic of Serbia, num. 18/2005) on planting material which has been passed in 2005 introduces 4 categories of planting material: prebasic, basic, certified and standard.

High-quality, healthy planting material is the result of the programme which comprises production, maintenance, multiplication and distribution of plant material intended to be marketed with indispensable official certification (label, certificate) as a warranty of the health status. The objective of certification is obtainment of true-to-type and pathogen-free planting material of prominent quality (Dulić-Marković 2003).

In recent years, massive dieback of fruitful plantings has been a serious problem in raspberry growing. With the exception of selection of inadequate parcels, some fungi from *Phytophthora* genus are major causal agents of the dieback (Duncan 2003). The symptoms of the disease are manifested in sudden and abrupt wilting and 'collapse' of canes at the close of spring and in the early summer (Koprivica et al. 2003).

Former import of uncertified planting material and its long-term propagation in the registered mother plantings has also largely influenced unfavourable circumstances in raspberry growing in our country. The imported planting material originates mainly from USA (Spooner Farm), Switzerland (Haberli), France (Marionnet) and Holland.

With regard to the importance of the raspberry production for Serbian fruitgrowing in general, the paper displays difficulties we encounter in the production of the planting material and methods we employ to overcome the problems.

Current state and difficulties in the production of raspberry planting material

As to achieve more efficient control of production of raspberry material Ružić et al. (2004) suggest the following measures:

1. Short-term measures – radical control measures of the current mother plantings for the presence of the phytopathogenic fungi and the control both of soils on which new mother plantings are established and the material with which the mother plantings are set up. Appropriately chosen parcels also play a distinctive role.
2. Long-term measures – production of the certified planting material and adequate registration of all planting material producers and importers, as well as organizing of the professional phytosanitary service on the national scale.

In compliance with the short-term measures, during 2004 the Ministry of Agriculture, Forestry and Waterpower Engineering of the Republic of Serbia introduced a project entitled 'The control of soft fruits mother plantings aimed at control of the *Phytophthora fragariae,* causal agents of root rot' the results of which point to a series of problems occurring in the production of raspberry planting material.

Inspection services of the Plant Protection Department of the Ministry have identified inadequate selection of parcels (heavy and impervious soils, inappropriate crop rotation), improper hygiene both of the workers and tools with which the operations of mother planting maintenance are carried out (transfer of workers and tools from infected to uninfected plantings) and incidence of phytopathogenic fungi *Phytophthora fragariae* var. *rubi* and *Verticilium* spp. as major causes of adverse circumstances. Laboratory analyses of the sampled mother plantings were conducted at the Fruit and Grape Research Centre in Čačak.

By the efficient organization of the national phytosanitary service import of infected planting material has been prevented on several occasions. Analyses have also been carried out at the Fruit and Grape Research Centre in Čačak, where, upon the analysis of the PCR products, a specific fragment of DNA of particular molecular mass (533 bp) was detected within the polyacrilamid gel, i.e. the incidence of the pathogenic fungus *Phytophthora fragariae* var. *rubi* was detected in the tested samples (Fig. 1).

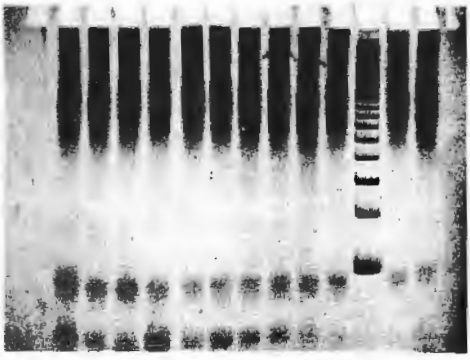
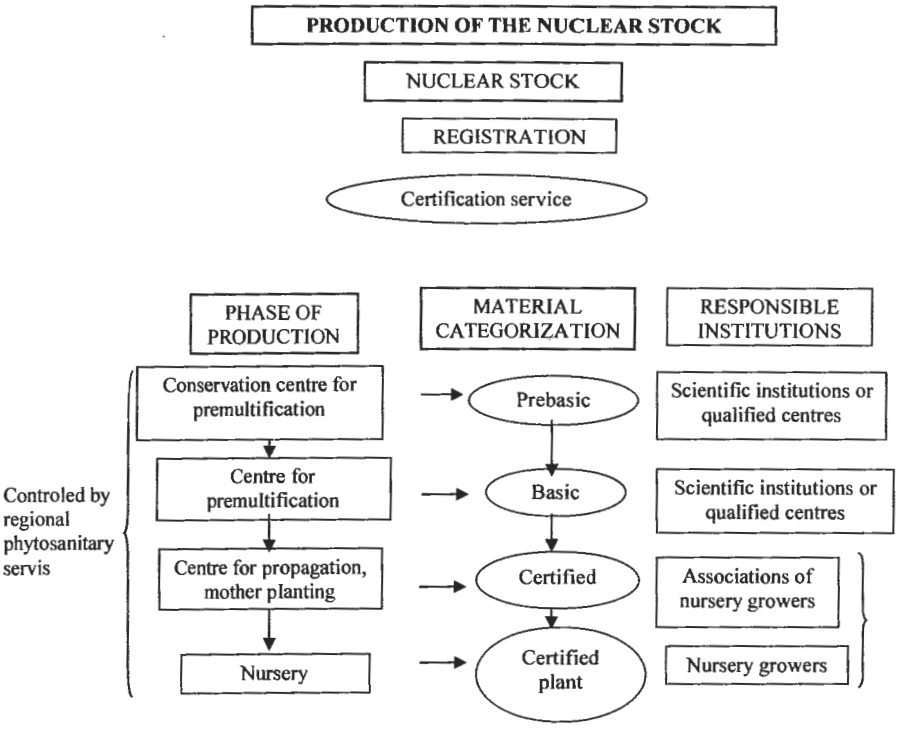


Figure 1. Detection of *Phytophthora fragariae* var. *rubi* by nested PCR

In view of the fact that the initial phases of certification are mainly related to scientific institutions which are provided with equipment for maintenance and testing of this kind of material under strictly protected conditions (screen houses), Ministry of Agriculture has assigned this activity to the Centre, and the stated shall be performed in two phases:
Phase 1 refers to the commencement of the production of planting material at the Centre with strict application of EPPO certification scheme (Scheme 1).



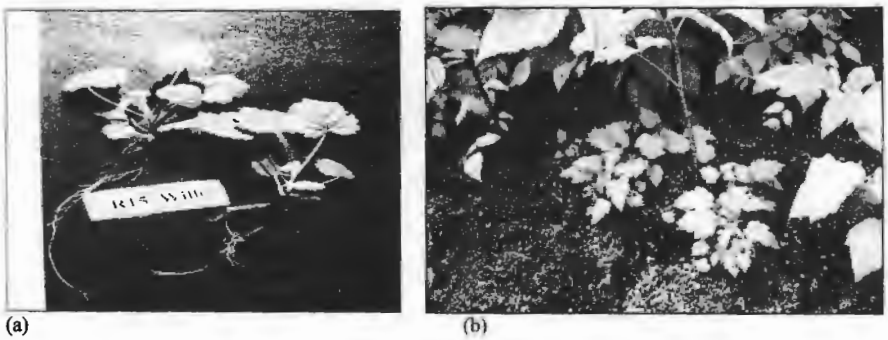
Scheme 1. Scheme of raspberry nursery stock certification

This scheme deals in detail with exact measures and procedures to be employed in the production of vegetatively propagated material of a particular plant whose health status is officially atested. Within typical certification scheme the certified material is propagated (through the exact number of steps) out of individual plants that were tested and found free from pests and diseases, and which were maintained and multiplied under the strictly controlled conditions, ensured from infection. Categories involved in raspberry certification system are as follows: nuclear stock, prebasic material, basic material, propagated material and certified material.

Selection of basic material necessitates defined methodology of clonal selection coupled with apropriate conditions for maintenance of the material. The selection is based on the following instances: pomological properties typical for the cultivar or the clone, quality and

cropping, uniformity of ripeness, appropriate vigour and no evidence of the symptoms of diseases (Paunović et. al. 2003). Micropropagation *in vitro* is the method of propagation of the basic material mostly applied. Conducted under fully controlled conditions along with permanent testing for the presence of viruses and *Phytophthora* sp. this method is assessed as the most reliable for obtainment of healthy raspberry planting material (Ružić and Lazić 2004).

According to OEPP standards micropropagation *in vitro* is indispensable and extremely important stage in the production of virus-free planting material of the *Rubus* genus. Since the micropropagation for commercial purposes is still an expensive method in our conditions, upon successful *in vitro* propagation and plant acclimatization (Fig. 2a), in order to reduce costs, we continued propagation in screen house in sterile substrate, in which 4-5 new shoots/plants have been obtained from each plant on average. Thus, a great number of new plants are induced in the base of a plant. (Fig. 2b). The plants are tested for the presence both of RBDV and NEPO viruses and *Phytophthora* prior to placement of explants and during multiplication *in vitro*, as well as upon multiplication *in vivo*. The results obtained have shown that obtained plants are healthy.



(a) (b)

Figure 2. Rooted plants *in vitro* are well developed, with numerous secondary roots (a); acclimatized plants transplanted to screen house (b)

The combination of *in vitro* and *in vivo* propagation methods, which we used for obtaining healthy planting material, proved to be more economic and faster than both micropropagation and standard raspberry propagation system by shoots (Milenković et al. 2005). Hence, micropropagation may be employed in the propagation of nuclear, prebasic and also basic material.

Nuclear stock is maintained under protected conditions, individually potted and raised on tables. Retesting is performed either annually or biennially, 0 being value of tolerance for the presence of pathogens.

Pre-basic material is obtained from the propagated nuclear material which is also maintained under protected conditions at minimal infection risk.

The first category planting material which is planted out is called basic material. One of prerequisites for satisfactory conditions, as regards the growing parcel, is its separation from other varieties of the *Rubus* genus by at least 1000 m. Basic material may be thus categorized as such for 4 years.

Mother plantings in which certified plants are propagated are established with basic material. Exploitation life of a mother planting is limited to 4 years, upon which period new mother plantings, based explicitely on the basic material, are set up.

In compliance with the stated procedure, during spring 2005, an area of 2.2 ha for propagation of raspberry basic material (cvs Willamette and Meeker) was established at the premises of the Centre (Fig. 3).

Figure 3. Raspberry mother planting – cvs Willamette and Meeker

The whole object is enclosed within a protective fence. During the planting and subsequent agro-technical activities the workers engaged in the activities followed basic recommendations in respect of measures proposed for a mother planting, both general and hygienic ones.

On establishment of the mother planting the following requirements were satisfied:

- The planting of container pooted plants in rows.
- Interrow spacing is 3 m.
- Physical separation from the uncertified *Rubus* spp. is at least 1,000 m.
- Soil testing for the presence of nematodes of virus vectors and *Verticillium* spp.

All through the vegetation cycle, the mother planting was continuously monitored for the presence and protection from the pests and diseases. At 20-day intervals the plants were tested for the presence of *Phytophthora fragariae* and other relevant viruses. All the analyses were carried out by the stated methods of detection and no pathogen or virus was detected on these occasions.

In the second phase of the project, planting material obtained from the propagation field shall be forwarded to the selected legal entities. The final product of the propagation in their nurseries shall be designated as raspberry certified material.

After the autumn of 2006, the planting material obtained from the propagation field of the Fruit and Grape Research Centre shall be used for the establishment of new certified mother plantings, whereas the material obtained from the nurseries of the selected legal entities shall be used for the establishment of the growing plantings.

Conclusion

Besides trueness-to-type of a cultivar, healthy planting material is a major precondition for a successful fruit production. Hence, production of certified raspberry planting material is initiated at the Fruit and Grape Research Centre. Produced under strictly controlled conditions and constantly tested for the presence of the pathogenic fungi and viruses, the planting material obtained shall be at disposal to the registered legal entities in the Republic of Serbia from the autumn 2005.

Overcoming the current difficulties which hamper the production of raspberry fruits, the national product of the Republic of Serbia, is possible only by obtaining sufficient numbers of certified plants and through continuous work on advancment of the growing technology.

Acknowledgements

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Production of certified healthy berry fruit plants in The Netherlands

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Abstract: High quality planting material of berry crops can be produced in strictly controlled certified propagation systems. The certification scheme that is in operation in The Netherlands for strawberry is presented in this article as an example. This system is based on the international EPPO standard for certification of strawberry. The basic principles of the scheme are explained, and attention is focussed on major attainments and pitfalls in the maintenance of such a system.

Key words: certification, diseases, legislation, pests

Introduction

In The Netherlands, certified planting material is produced in fruit crops like apple, pear, strawberry and raspberry. On the one hand, this material needs to meet all the criteria listed down in EU legislation (EU Marketing Directive 92/34/EC and EU Phytosanitary Directive 2000/29/EC) in order to realise free movement of planting material within the community. On the other hand, fruit growers want to have at their disposal planting material of the highest quality possible. It is not necessarily the requirements laid down in the EU-directives that are the only quality criteria for professional fruit growers.

Quality is not only about diseases and varietal purity, but perhaps equally important for the fruit grower is the pomological quality of the planting material. Is the planting material true to type, is it the best selection available? It is important for growers to be able to recognize high quality planting material in the market. To this end, unequivocal labelling of plants, covering all the aspects mentioned above, must be part of any effective certification program.

In The Netherlands, certification schemes are in operation, covering not only the requirements listed in the two EU directives, but also paying attention to trueness to type, pomological quality, affiliation of the planting material, and external quality of the plants-to-be-sold. The certification schemes were built on the basis of the certification schemes published by OEPP/EPPO in the last two decades. The schemes are designed around three principles; 1. complete testing of starting material (Nuclear Stock), 2. limited number of generations of propagation, and 3. requirements for isolation and inspection for every generation. The certification inspections in The Netherlands are organised in such a way, that inspection activities cover both the EU-requirements and the typical certification-requirements in relation to plant quality etc. When planting material meets all the requirements, it is labeled with an official Naktuinbouw-label, which is a plant passport and a 'quality-plus' label in one. The approach is illustrated by a more detailed presentation of the Naktuinbouw strawberry certification scheme.

Pests and diseases spreading with propagation material of strawberry

Many virus, phytoplasma, bacterial and fungal diseases can spread with strawberry propagation material. Furthermore, pests like strawberry mite may hide in plants used for planting. Examples of pests and diseases spreading worldwide with propagation material are tomato ring spot virus, strawberry crinkle virus, strawberry mottle virus, *Colletotrichum acutatum* (the causal agent of anthracnose), *Phytophthora fragariae* (the causal agent of red stele), *Verticillium dahliae* (the cause of *Verticillium* wilt), *Aphelenchoides fragariae* (a foliar nematode causing spring dwarf) and *Phytonemus pallidus* (strawberry mite). An overview of the most important pests and diseases that might spread with propagation material is presented in Table 1.

Certified production of strawberry

A successful system for the production and certification of healthy planting material ideally should be built on three principals; maximum test effort in the starting material, a limitation of the number of generations, and production under strict isolation and inspection requirements.

Testing

The basis of any propagation system is starting material, which is tested for any disease known to occur in the crop under consideration, in this case strawberry. Testing for viruses usually is covered by the use of an extensive set of different kinds of methods; sapinoculation on herbaceous indicator plants, leaf grafting on *Fragaria vesca* indicators, ELISA and PCR. For the detection of fungi traditional plating techniques may be used (*Verticillium* spp. and others), and some of the fungi ate tested for by special techniques like baiting (in the case of *Phytophthora fragariae*), or a paraquat test (in the case of *Colletotrichum acutatum*). For the detection of bacteria modern techniques like Bio-PCR may be used, and the presence of nematodes is checked in a number of traditional nematode tests. Infected plants may be cleaned by using combinatory techniques like heat treatment and meristem tip culture. The whole sanitation and testing procedure may take one growing season at least. The healthy starting material normally will be denominated as nuclear stock or pre basic material.

Table 1. Overview of the major pests and diseases worldwide that may spread with propagation material of strawberry.

Viruses spread by aphids	strawberry crinkle virus
	strawberry mottle virus
	strawberry latent C virus
	strawberry mild yellow edge virus
	strawberry veinbanding virus
	strawberry pseudo mild yellow edge virus
Viruses spread by nematodes	tomato ringspot virus (*Xiphinema* spp.)
	tomato black ring virus (*Longidorus* spp.)
	raspberry ringspot virus (*Longidorus* spp.)
	strawberry latent ringspot virus (*Xiphinema* spp.)
	arabis mosaic virus (*Xiphinema* spp.)
	tobacco rattle virus ((*Para-*)*trichodorus* spp.)

Viruses spread by fungi	tobacco necrosis virus (*Olpidium brassicae*)
Virus-like agents	fragaria chiloensis virus tobacco streak virus strawberry pallidosis chlorotic fleck strawberry feather leaf strawberry leaf roll vein yellowing
Phytoplasmas	strawberry aster yellows green petal strawberry lethal decline bronze leaf wilt phytoplasma (mycoplasma) yellows multiplier disease witches' broom
Bacteria	rickettsia yellows marginal chlorosis *Xanthomonas fragariae* *Xanthomonas arboricola* var. *fragariae*
Fungi	*Phytophthora fragariae* var. *fragariae* *Phytophthora cactorum* *Colletotrichum acutatum*, *Verticillium dahliae* *Verticillium albo-atrum* *Rhizoctonia fragariae*
Foliar nematodes	*Aphelenchoides besseyi* *Aphelenchoides blastophthorus* *Aphelenchoides fragariae* *Aphelenchoides ritzemabosi* *Ditylenchus dipsaci*
Virus vector nematodes	*Xiphinema americanum* (tomato ringspot virus) *Xiphinema diversicaudatum* (Arabis mosaic virus and strawberry latent ringspot virus) *Longidorus macrosoma* (raspberry ringspot virus) *Longidorus attenuatus* (tomato black ring virus) *Longidorus elongatus* (raspberry ringspot virus and tomato black ring virus) (*Para*)-*trichodorus* spp. (tobacco rattle virus)
Arthropods	*Chaetosiphon fragaefolii* (strawberry aphid) *Phytonemus pallidus* (strawberry mite)

Generations
Planting material will then be propagated in a system limiting the number of generations in order to minimise the risks of re-infestation with serious pests and diseases. Regular checks are performed on the pomological quality and the trueness to variety of the plants. In The Netherlands a system is in operation that is based on international standards, published by OEPP/EPPO (OEPP/EPPO 1994). The number of generations is limited to four. The different steps are identified and labelled differently; SEE, SE, EE and E, respectively.

Isolation and inspection
For the different steps in production, isolation and inspection requirements are set, that are laid down in a set of official documents. The requirements are very strict at SEE level, and become less strict coming down the line via SE and EE, ending with E. There are requirements in relation to insect vectors (aphids), nematodes (sampling), the distance to neighbouring plots etc. All steps of production are under continuous control of inspectors.

Major attainments and pitfalls

By operating a strictly controlled propagation system, it has been shown in the last decades that it is possible to produce healthy planting material of berry crops like strawberry to a very high standard. This is an important attainment in itself for professional berry growers, who only want to use the highest quality planting material available. But it has been shown to be possible to meet European legal standards in such a propagation scheme as well. Requirements laid down in the Marketing Directive 92/34/EC and the Phytosanitary Directive 2002/29/EC have been incorporated in the Dutch certification scheme, so that growers are faced only with one integral set of inspection and certification standards.

Running a certification scheme that keeps up with changes in international phytosanitary legislation, and fulfilling the quality requirements of growers at the same time, is not always an easy job to do. Many challenges have to be faced, of which only two are mentioned here:

An important disease in strawberry is *Xanthomonas fragariae*, the causal agent of angular leaf spot. Very sensitive methods are available for the detection of this bacterium. The disease is common in strawberry worldwide, widespread in Europe, but still has a quarantine status. Little is known about its life cycle, and major ways of infection are unknown. The relation between detected amounts of bacterium and the risk of disease development is unclear. The trade of high grade strawberry material may be seriously hampered by unjust interceptions or destruction of plants in trade, as a result of the use of very (too?) sensitive tests performed on the basis of legislation in the present situation where basic knowledge about the pathogen is lacking.

Another example of a problem that has to be handled is the detection of *Colletotrichum acutatum*. It has a quarantine status in strawberry, but is widespread in a number of other agricultural crops, as well as in nature. As is the case with *Xanthomonas fragariae*, basic knowledge about the biological behaviour of the pathogen is not available. It is completely unknown how important the different ways of infection are. Spreading the disease with high grade certified planting material might well be a negligible risk. Free trade in Europe of high standard healthy material of strawberry may practically be made impossible once sensitive molecular detection becomes the standard method for importation checks, and alternative planting material may not be available.

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The use of compost and green manure to control soil borne diseases of strawberry

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Abstract: The control of soil borne diseases, mainly *Verticillium* wilt, by planting strawberries (cultivar Elsanta) in raised beds amended with windrow compost or after the cultivation of different green manures was tested in two field experiments. In 2002, a first experiment included the use of windrow compost and leaves of annual wormwood (*Artemisia annua*), a medicinal plant containing anti-microbial compounds. Planting in raised beds with compost, alone or mixed with soil, resulted in a significantly higher yield than planting in beds with just soil. The amendment of the soil with compost also increased the soil microbial activity. This stimulation of soil microorganisms had a beneficial effect on the strawberry crop as yield was significantly correlated to soil microbial activity. In 2003, a plot with a high population of *Verticillium* spp. was planted with red clover (*Trifolium pratense*), rye (*Secale cereale*) and brown mustard (*Brassica juncea*) before the planting of strawberries. Young and old windrow compost were also included in this trial. None of the treatments had a significant effect on disease incidence and yield. However, the number of microsclerotia of *Verticillium* spp. in the soil was significantly reduced by the brown mustard green manure. Because of the very high population level of the entire experimental plot with *Verticillium* spp., this reduction did not result in a lower mortality or higher yield than the control treatment.

Key words: biofumigation, soil microbial activity, *Verticillium* wilt

Introduction

The production of strawberry (*Fragaria* x *ananassa*) can be limited by different soil borne diseases such as red stele (caused by *Phytophthora fragariae* var. *fragaria*), crown rot (caused by *Phytophthora cactorum*), verticillium wilt (caused by *Verticillium dahliae* and *V. albo-atrum*) and black root rot (caused by several pathogenes including *Rhizoctonia* spp. and *Pythium* spp.) (Ellis et al. 1997). Two important means to control soil borne diseases are the use of resistant cultivars and treatment with fungicides before or after planting. However, the utilisation of cultivars resistant to soil borne diseases can be restricted by other traits such as quality, yield or resistance to pests or airborne diseases. Limitations to the use of fungicides are high costs, limited efficacy in the soil and resistance of the pathogens. Therefore, research on the use of cultural methods to control soil borne diseases is needed.

Several years ago, the use of cultural methods to control *Phytophthora* root rot of red raspberry (*Rubus idaeus*) caused by *Phytophthora fragariae* var. *rubi*, was tested successfully in Switzerland (Neuweiler & Husistein 2000, Ançay & Michel 2004). The two most important cultural improvements were planting on raised beds and the use of compost. The beneficial effect of compost on plant health has also been demonstrated for other plant-pathogen complexes (Fuchs 2002). Therefore, we wanted to test the effect of compost on soil borne diseases of strawberry.

The other cultural approach we wanted to test was the use of specific green manures. Legumious species have proved to be efficient to control root rot of cinchona (*Cinchona*

succirubra) caused by *Phytophthora cinnamoni* (Werner Heller, pers. comm.). Recently, a technique using glucosinolate-containing green manure crops to control a range of soil borne diseases has been developed (Kirkegaard & Matthiessen 2004). When incorporating such green manure crops into the soil, glucosinolates are decomposed to several molecules, including isothio- and thiocyanates. The latter ones are volatile substances that are toxic to a range of soil microorganisms, including several soil borne pathogens. This technique, which is named biofumigation, is an alternative to methyl bromide to control soil borne pathogens and pests. The use of medicinal plants to control plant diseases was recently investigated in Switzerland as an alternative to the use of copper-based fungicides to control late blight of potato in organic farming (Bassin & Forrer 2001). Annual wormwood (*Artemisia annua*) was one of the plants having an *in vitro* activity against *Phytophthora infestans*, the causal agent of late blight (Pia Malnoë, pers. comm.).

Material and methods

We tested windrow compost, red clover (*Trifolium pratense*), rye (*Secale cereale*), annual wormwood (*Artemisia annua*), and brown mustard (*Brassica juncea*), the latter a biofumigation species, in two field experiments for their use to control soil borne diseases of strawberries.

Experiment A

In summer 2002, strawberries (cultivar Elsanta) were planted at the Domaine de Bruson, an experimental site belonging to Agroscope Changins Wädenswil – Centre des Fougères, situated at 1080 m.a.s.l. near the Grand St-Bernhard (Valais, Switzerland). The experimental field was naturally infected with *Verticillium dalhiae* (at a low level) and an unidentified pathogen causing black root disease. Each experimental plot consisted of one row of 6 m length planted with 24 strawberry plants (25 cm within row and 1.0 m between rows). The experimental layout was a randomized complete block design (RCBD) with 4 replications. The cultural control methods tested are given in Table 1. The mature windrow compost and annual wormwood leaves were amended to the soil just before planting the strawberries. Beds were 10 – 15 cm high and 40 cm wide and were covered with black polyethylene plastic mulch. Plants were drip irrigated and standard fertigation was applied in 2003. Leaf diseases and pests were controlled by standard fungicide and insecticide applications. The experiment ended after fruits were harvested in summer 2003.

Experiment B

The second experiment took place in a plot highly contaminated with *Verticillium* spp. The experimental layout was a RCBD with 4 replications, each experimental plot measured 5 x 1.5 m. The experiment started with the planting of brown mustard in August 2003 (Table 1). In October, this biofumigation crop was mulched at the flowering stage and incorporated into the soil which was left fallow during winter season. Red clover and rye were sown in autumn 2003. Because of a poor seed quality, emergence was very low and both species were resown in spring 2004. In April, both green manures were mulched and incorporated into the soil. At the same time, the two composts were added and mixed with the soil. Both composts were windrow compost, produced at the same site but different in age (Table 2). Two weeks later, 20 strawberry plants (cultivar Elsanta) were planted in one raised bed per experimental plot with a within row distance of 25 cm. Plants were drip irrigated but no fertigation was applied. P_2O_5, K_2O and Mg fertilizer was added before planting with the exception of the two compost treatments. Only the nitrogen fertilizer treatment received nitrogen in form of ammonium nitrate, split in three doses of 28 kg N/ha in summer 2005. Leaf diseases and pests were

controlled by standard fungicide and insecticide applications. The experiment ended after fruits were harvested in summer 2005.

Table 1. Cultural methods tested in two field experiments (A and B) to control soil borne diseases of strawberry.

Experiment	Treatment	Remarks
A	Raised beds, soil	
	Raised beds, soil and compost	105 m^3/ha of compost
	Raised beds, compost	105 m^3/ha of compost
	Raised beds, soil and annual wormwood	250 kg/ha of dried leaves of annual wormwood
	Control	Beds not raised
B	Old compost (4 months)	105 m^3/ha of compost
	Young compost (6 weeks)	105 m^3/ha of compost
	Red clover	
	Rye	
	Brown mustard	Cultivar ISCI-20
	Nitrogen fertilizer	84 kg N/ha
	Control	

Analysis of soil parameters

Soil microbial activity was measured in both experiments using the FDA method (Schnürer & Rosswall 1982). Soil samples for FDA analysis were taken in experiment A in May 2003 and in experiment B in May 2004, May 2005 and August 2005.

The number of *Verticillium* spp. microsclerotia in the soil was determined in experiment B using Sorensen's NP-10 selective medium (Butterfield & DeVay 1977, Kabir *et al.* 2004). One soil sample per experimental plot was taken in May 2005 and air-dried for six weeks at room temperature. Per sample, five 100 mg aliquotes were dry-plated on the NP-10 medium using an Anderson sampler similar device. After two weeks of incubation at 24°C, soil was removed from the medium surface and the number of *Verticillium* spp. microsclerotia was counted under a dissecting microscope.

Table 2. Description of the two windrow composts used in experiment B.

	Old compost	Young compost
Organic matter	44.0%	40.5%
Dry matter	58.4%	62.8%
C/N ratio	13.1	24.3
Specific weight	571 kg FM/m^3	362 kg FM/m^3
pH (H_2O)	8.0	7.5
Salinity (H_2O)	3.21 m-S/cm	2.21 m-S/cm
Nitrogen	19.5 kg/t DM	9.6 kg/t DM
Ammonium (NH_4-N)	0.62 kg/t DM	0.30 kg/t DM
Phosphorus (P_2O_5)	8.2 kg/t DM	4.0 kg/t DM
Potassium (K_2O)	14.7 kg/t DM	6.2 kg/t DM
Calcium (Ca)	63.8 kg/t DM	79.3 kg/t DM
Magnesium (Mg)	6.6 kg/t DM	6.4 kg/t DM

Results and discussion

Experiment A

Yield was significantly higher in the two treatments with compost (Figure 1). The slightly higher yield of the control treatment compared to raised beds of soil alone was most probably caused by the dry spring and summer in 2003 combined with a shortage of irrigation. In this situation the drought stress was higher for the strawberries planted in raised beds compared to the ones planted in plain soil. No significant differences occurred between the treatments concerning the plant vigour and disease incidence.

A significant correlation existed between the yield and the soil microbial activity (Figure 2). Higher yield was positively correlated with a higher general activity of the soil microorganisms. The positive role of an active soil life, as promoted by organic growers, has been reported for *Pythium* root rot of poinsettia (Boehm & Hointink 1992). The disease was suppressed by a high microbial activity in the potting mix even though the *Pythium ultimum* population did not decline.

Experiment B

No influence of the treatments on the *Verticillium* wilt incidence and on the yield was observed (Table 3). However, the biofumigation treatment using brown mustard significantly reduced the number of *Verticillium* spp. microsclerotia compared to the control treatment (Figure 3). This relatively small decrease of 25% of the *Verticillium* population can partly be explained by a late incorporation in soil of brown mustard in October when the soil was already relatively cold. The chemical reaction creating the isothio- and thiocyanates would then be relatively slow, reducing the biofumigation effect against *Verticillium* spp. The second treatment that slightly reduced the number of microsclerotia was the old compost. In contrast, the young compost had no influence

on the number of microsclerotia in the soil. The biological control mechanisms of composts are multiple, and the age and stability of the composts are important factors for their utilisation in the control of soil borne diseases (Hoitink et al. 1997). The younger compost enhanced the microbial activity of the soil slightly. This activity is important for the control of *Pythium* and *Phytophthora* diseases (Hoitink et al. 1997), but is probably of lesser importance in the control of *Verticillium* wilt. In fact, rye, the treatment which most stimulated the microbial activity of the soil, had no effect on the number of microsclerotia in the soil (Figures 3 and 4).

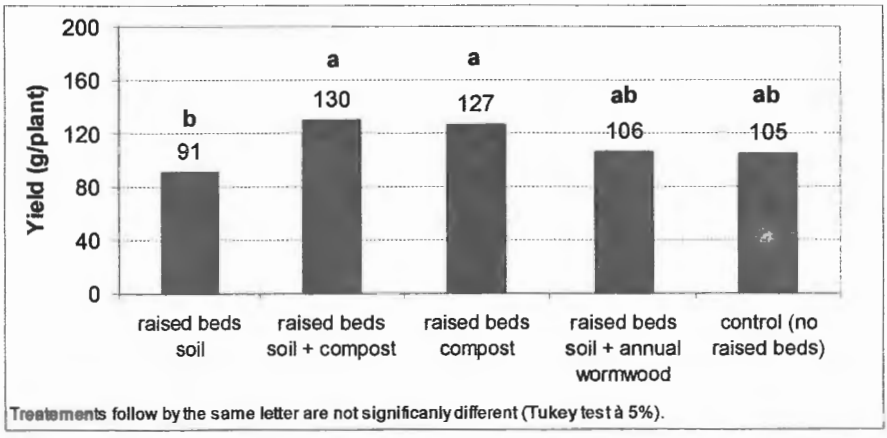

Figure 1. Yield (first grade) of experiment A in summer 2003.

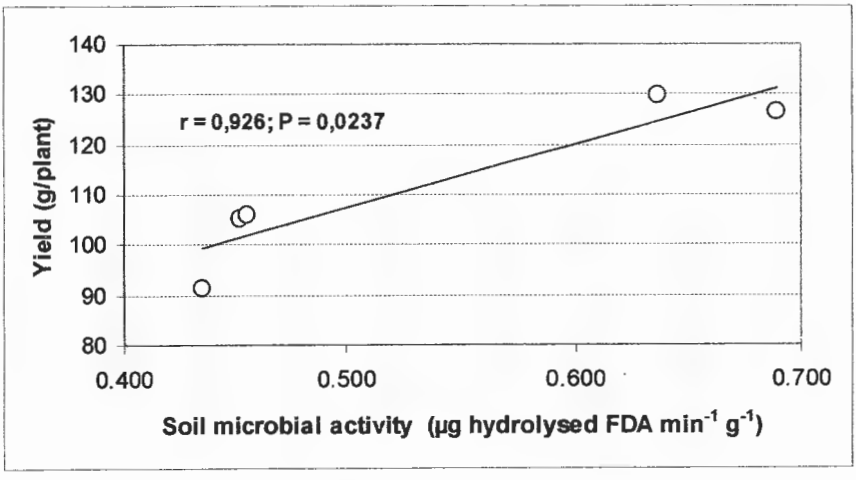

Figure 2. Correlation between soil microbial activity and yield (first grade) in experiment A. The two treatments with the highest microbial activity are the two compost-amended treatments.

Table 3. *Verticillium* wilt incidence and yield in experiment B. There were no significant differences among treatments for any of the two parameters shown.

Treatment	*Verticillium* wilt (incidence)	Yield, first grade (g/plant)
Old compost	60%	121
Young compost	61%	133
Red clover	74%	130
Rye	64%	118
Brown mustard	57%	98
Nitrogen fertilizer	69%	139
Control	74%	124

Conclusions

The most promising means of control of *Verticillium* wilt is the biofumigation using special cultivars of brown mustard. Unlike the other green manure corps, which mainly activate the microbial activity, brown mustard results in the production of volatile and toxic substances that are lethal to certain soil pathogens, including *Verticillium* spp. (Down *et al.*, 2004). The use of old, mature compost has also some control potential. Field trials combining both methods are ongoing to check if such a combination may improve the control of soil borne diseases of strawberries.

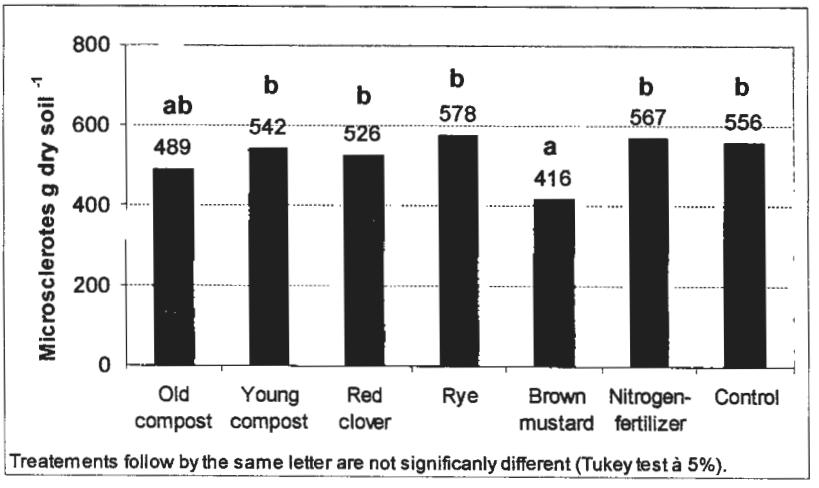

Figure 3. Number of microsclerotia of *Verticillium* spp. in the soil of experiment B in May 2005.

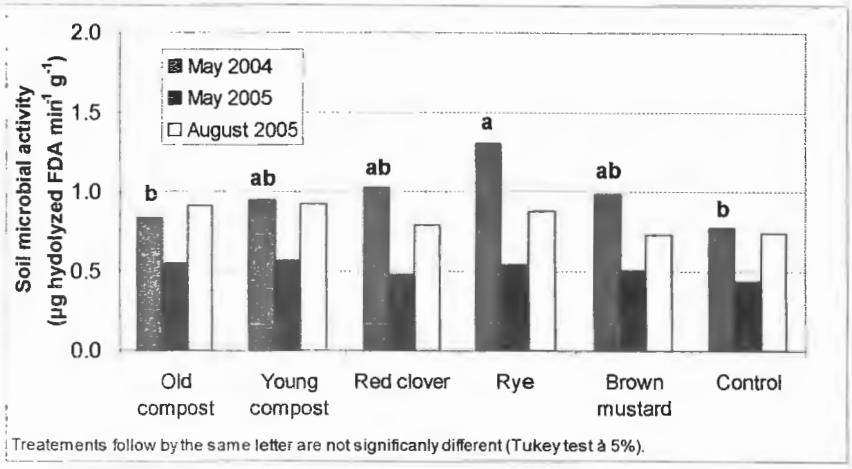

Figure 4. Soil microbial activity of experiment B. Differences between treatments were significant in May 2004, but not in May and August 2005.

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Epidemiology and control of cane blight (*Leptosphaeria coniothyrium*) of raspberry

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Abstract: Preliminary observations on cane blight development in canes artificially wounded between July and early October 2004 showed that most infection occurred in July / August, but that infection with cane blight was still occurring in early October. Most infection occurred in wounds made at the base of the cane, but a low percentage of wounds made at points around 1.5-2.0 m above the ground also became infected with cane blight, which is difficult to explain on the basis of existing knowledge on cane blight epidemiology. This study will be repeated in the second year of the project. In a replicated trial in an open field raspberry plantation of cv Glen Ample nine different fungicide products were compared for their effectiveness in controlling cane blight in comparison to an untreated. Treatments were applied post harvest at the end of picking in July 2004 and repeated two weeks later. Fungicide efficacy was assessed the following June (2005) as percentage dead or dying fruiting canes. The percentage of canes with cane blight lesions was recorded on the fruiting canes after harvest in September. None of the treatments tested gave complete control of cane blight, although it was not possible to distinguish between cane death due to cane blight and that due to 'wet feet' or *Phytophthora* root rot which were also present in the plantation. Occidor (carbendazim) was the most consistently effective product. Folicur (tebuconazole) and Signum (pyraclostrobin + boscalid) were also effective. These fungicides will be further evaluated in 2005.

Key words: raspberry, fungicide, cane blight

Introduction

Cane blight, caused by the fungus *Leptosphaeria coniothyrium*, can result in significant losses in raspberries and other cane fruit, particularly in seasons following wet harvests. The fungus overwinters on old cane debris, and spores produced in spring and summer during rain infect the base of the young cane through wounds. The wounds can be due to a variety of causes, including frost, mechanical injury or insect feeding e.g. by cane midge larvae (*Resseliella theobaldi*). The disease develops over the winter and, once the cane is girdled, the fruiting cane wilts and dies the following summer, usually just as fruiting begins. No new studies have been conducted on cane blight since the initial work on epidemiology, fungicides and disease management was done in Scotland at SCRI in 1970s and 1980s. These studies showed that weather, variety and cane maturity were important factors in determining the severity of cane blight damage. In Scotland the incidence of cane blight was greatest in plantations that were machine harvested due to damage to the primocanes by the picking machinery (Williamson & Hargreaves, 1978). In other plantations the cane blight fungus gained entry through wounds on the primocanes caused by old cane stubs, strimmer damage during cane thinning and through damage caused by cane midge larvae feeding and frost. Seemuller et al (1988) showed that, in studies in Germany, the cane blight fungus could invade healthy undamaged cane, but the invasion progressed very slowly and was enhanced by weakening of the canes

by defoliation. Cane blight is present in the USA, but appears to be much less damaging, even in machine harvested crops. Reasons for the difference are not clear. Other studies outside the UK on cane blight have been very limited and not added much to the earlier studies at SCRI.

For the last ten years cane blight has been of little importance in plantations in the UK mainly because of the widespread planting of varieties such as Leo that are less susceptible, the use of organophosphate insecticides to control cane midge and the availability of effective fungicides. During 2003 considerable numbers of fruiting canes were either severely debilitated or died as the result of cane blight infection in many summer fruiting raspberry plantations in England and Wales, most notably those of the cultivar Glen Ample. Cane and as a consequence yield loss due to this disease was observed not only in open field but also plantations which had been protected in the current and previous year by Spanish Tunnels during harvest, or from pre flowering until the end of harvest. Where cane blight has occurred in other cultivars, it has usually been associated with obvious damage to canes. The problems observed in Glen Ample have been more difficult to explain as there has not been obvious damage on canes for the fungus to enter. Changes in raspberry production methods, including production under polytunnels and different methods of spawn control, including use of paraquat which could damage young spawn or even encourage the cane blight fungus to sporulate on cane stubs, may be responsible for the reappearance of the disease. In addition, seasonal weather conditions have changed. With the extension of warmer conditions into October and milder winters, the period for fungal activity has been extended and such conditions may delay cane maturity, extending the period of cane susceptibility to the disease.

Carbendazim is currently the only known product to effectively control cane blight and, in the UK, its use is permitted post harvest on cane fruit crops. This restriction of usage to the post harvest period is not ideal for effective control. There are also concerns over the use of carbendazim by consumers. The availability and usage of carbendazim on raspberries and other cane blight-susceptible cane fruit crops is therefore likely to be limited in the future. Alternative products such as diclofluanid or thiram were shown in earlier trials to be ineffective against this pathogen. There are now many new fungicide products available, some of which may be effective against this disease and could be used to replace carbendazim.

The objectives of this study, funded by the Horticultural Development Council, were to (a) re-examine the epidemiology of cane blight in relation to changes in variety, production methods and seasonal weather and (b) evaluate recently introduced fungicide products for efficacy against this disease and identify an acceptable effective alternative to carbendazim. Preliminary results of this work are presented here.

Materials and Methods

Cane blight monitoring

In 2004 one plantation of Glen Ample, where cane blight had previously been a problem was chosen for the study. The site was located on a commercial Farm in Addlestone, Surrey, in a 6-7 year old plantation of Glen Ample. The raspberry plantation received a standard programme for pest and disease control, including three sprays of carbendazim for cane blight control applied post harvest at 10-14 day intervals from early August. Old fruiting canes were pruned out and pulverised in the first week of August.

The plantation was visited fortnightly from 13 July until 2 October 2004. At each visit 30 primocanes were tagged and their rind damaged at heights of 0.3 (zone 1) and 0.6m (zone 2) above the ground up to zone 5. The canes were selected at random, across the rows and were at least 1 m away from the next one to be sampled, two new rows being used as a source of

sample canes for each visit to the site. Initially, 14 days after wounding the canes were cut at ground level, their tips and leaves removed and then sent to East Malling Research so that the artificial lesions could be examined in the laboratory for cane blight infection. From 5 August onwards canes were harvested one month after damaging to allow the cane blight to develop before being despatched for assessment. Canes were assessed for cane blight infection by scraping off the rind and checking for lesion development associated with the damaged areas. Cane blight was confirmed by damp incubation of canes with suspect lesions under UV light to encourage sporulation.

Fungicide evaluation

The site was located in a mature open field plantation of cv Glen Ample on a commercial farm at Taplow, Berkshire, where cane blight was known to be a problem. Each plot was 10 m (approximately 20 stools) in length with 5 m between plots in the same row and separated from plots in adjacent rows by 2.2 m. Each treatment was replicated four times in a randomised block design.

The products listed in Table 2 were evaluated in 2004 at the recommended dose. An untreated control was included. Treatments were applied at 500 L/ha using a CP15 knapsack sprayer. Sprays were directed to the bottom metre of the primocane only. Treatments were applied on two occasions – immediately post harvest on 27 July with a second spray two weeks later on 10 August. All plots were treated routinely for control of pests and other diseases as needed.

In June 2005 the plots were assessed visually for signs of cane blight by recording the number of dead or dying canes in alternate stools in each plot. As soon as harvest was complete in early August 2005, all the canes in each plot (approximately 50-100 canes per plot) were cut off at ground level and taken back to the laboratory for assessment for cane blight. For assessment each cane was divided into two zones – zone 1 (bottom 1 metre of cane) to which the treatments had been applied and zone 2 (rest of cane above 1 metre in height) that had not been sprayed. The outer rind of the cane was scraped off and the canes assessed for presence or absence of cane blight lesions, extent of girdling and presence of pycnidia of *Leptosphaeria coniothyrium* (Williamson & Hargreaves 1981).

Results and discussion

Cane blight monitoring

The percentage of wounds that became infected with cane blight at different heights (zone 1-5) is shown in Table 1. Rainfall data from the nearest weather station (RHS Wisley, approximately 10 km South West of Addlestone) for the week prior to wounding and the time between wounding and harvest is also given in the Table. The greatest percentage of wounds that became infected was in the lower part of the cane (base and zone 1) and infection decreased the higher up the cane the wounds had been made. However, infection still occurred in wounds made up to 1.5-2 m above the ground. Currently cane blight infection is thought to arise from rain splash of conidia from pycnidia on cane debris or old pruning stubs at the base of the cane. On this basis it is difficult to explain how infection arose high up the cane.

Table 1 Cane blight monitoring – Time of cane wounding, date canes harvested and % wound areas infected with cane blight. Rainfall data is from RHS Wisley. The canes were treated with carbendazim 10 August, 1 September and 7 October.

Damage date	Date harvested	% wounds infected with cane blight						Mean % infected	Rain mm in 7 days before wounding	Rain mm between damage and harvested
		Base*	Zone 1	Zone 2	Zone 3	Zone 4	Zone 5			
13 July	**23 July**	-	10.7	14.3	0	3.6	3.6	6.4	29.7	0.6
23 July	**5 August**	73.3	53.3	60.0	50.0	3.3	-	48.0	0.6	8.8
5 August	**3 September**	-	69.7	39.4	30.3	12.1	9.1	32.1	5.4	61.6
19 August	**17 September**	3.4	34.5	34.5	20.7	3.4	-	19.3	48.8	22.8
16 September	**26 October**	5.7	42.9	0	42.9	0	-	18.3	14.0	100.2
2 October	**26 October**	11.1	22.2	7.4	3.7	7.4	-	10.4	5.8	89.6
	Mean	15.8	38.9	25.9	24.6	5.0	2.1			

* Wounds were not made at the cane base, so cane blight recorded at the base is most likely to have arisen from natural wounds

It is possible that the perithecial state of the cane blight fungus may be present on the old cane debris and this infection resulted from ascospores which tend to be explosively released from perithecia and therefore have more opportunity to be carried higher up the cane than the splash dispersed conidia. This possibility will be explored in the second year of the project.

The greatest number of wounds infected occurred in late July / early August. This did not appear to be closely related to rainfall as it did not coincide with the highest rainfall (Table 1). Infection still occurred at the later wounding dates but at a much lower incidence. This may be related to cane maturity as the fungus is less able to invade mature canes or could be related to release of inoculum as the highest incidence of wound infection also coincided with pruning out and pulverising of the old fruiting canes. Application of carbendazim post harvest for cane blight control (Table 1) did not appear to have much impact on wound infection. In 2005 the monitoring of disease development will be repeated and extended to five sites.

Fungicide evaluation

The interpretation of the results was complicated by cane death due to 'wet feet' (waterlogging) that was prevalent in one large patch in the plantation and by *Phytophthora* root rot (*Phytophthora fragaria* var. *rubi*) that was also present in one distinct patch in the plantation but also scattered at lower incidence throughout the area. This was taken into account in the statistical analysis of the data. The data for percentage dead or dying canes are presented in Table 2. Numbers of dead or dying canes were significantly reduced, compared to the untreated control, in plots treated with Occidor (carbendazim), Folicur (tebuconazole) or Signum (pyraclostrobin + boscalid). Other fungicide treatments, apart from Elvaron Multi and Talat, also reduced the number of dead or dying canes, but these differences were not significant. The percentage of canes with basal lesions (Table 2) was also reduced by these fungicides but none of the treatments were significantly different from the untreated. Many of the cane blight lesions observed were actively sporing. These were located at any height on the canes with many up to 2 metres above the ground. Since the treatments were only applied to the bottom metre of cane, assessment of sporing cane blight lesions was divided into zone 1 (bottom metre of cane) and zone 2 (> 1 metre above the ground). As expected, there was no effect of treatment on percentage canes with sporing lesions on the top section of cane. The percentage of sporing lesions in the bottom one metre was significantly reduced in plots treated with Occidor (carbendazim). Folicur, Amistar and Signum also reduced the percentage of canes with sporing lesions but differences were not significant.

None of the treatments tested gave complete control of cane blight, although it was not possible to distinguish between cane death due to cane blight and that due to 'wet feet' or *Phytophthora* root rot which were also present in the plantation. Occidor (carbendazim) was the most consistently effective product. Folicur (tebuconazole) and Signum (pyraclostrobin + boscalid) were also effective. Most of the other fungicides reduced cane death compared to the untreated, but differences were not significant. Elvaron Multi and Talat appeared to be the least effective of the products tested.

Table 2 Effect of fungicide treatments applied in 2004 on incidence of dead / dying canes assessed in June 2005 and incidence of cane blight lesions at various cane heights in the plantation assessed post harvest in September 2005. Data presented is angular transformation of the original. The back transformed percentage figures are given in parentheses.

Product	Active ingredient	Rate product / ha	Mean % dead or dying canes		% canes with basal lesions (zone1)		% canes with sporing lesions (zone 1)		% canes with sporing lesions (zone 2)	
Untreated	-	-	36.3	(35.0)	41.6	(44.1)	28.6	(22.9)	15.5	(7.2)
Occidor	carbendazim	1.1 L	**22.0**	(14.0)	27.9	(21.8)	**14.9**	(6.6)	12.4	(4.6)
Folicur	tebuconazole	1.0 L	**20.8**	(12.6)	30.9	(26.4)	18.8	(10.3)	7.8	(1.8)
Amistar	azoxystrobin	1.0 L	25.8	(19.0)	28.0	(22.0)	18.8	(10.4)	17.3	(8.8)
Elvaron Multi	tolylfluanid	3.4 kg	37.4	(36.8)	42.2	(45.1)	22.8	(15.0)	16.8	(8.3)
Talat	tolylfluanid + fenhexamid	3.0 kg	32.0	(28.0)	30.0	(24.9)	23.6	(16.1)	21.8	(13.8)
Signum	boscalid + pyraclostrobin	1.8 kg	**18.6**	(10.1)	33.5	(30.4)	20.8	(12.7)	13.2	(5.2)
Frupica	mepanipyram	0.8 kg	24.8	(17.6)	49.3	(57.5)	29.7	(24.5)	18.1	(9.6)
Switch	cyprodonil+fludioxonil	1.0 kg	26.4	(19.7)	38.8	(39.3)	27.0	(20.6)	18.8	(10.4)
Scala	pyrimethanil	2.0 L	26.0	(19.2)	32.8	(29.3)	21.4	(13.3)	13.9	(5.8)
Treatment F probability			**0.031**		**0.086**		**0.244**		**0.695**	
LSD			**11.7**		**14.8**		**11.6**		**13.8**	
df			**26**		**27**		**27**		**26**	

Figures in bold are significantly different from the untreated

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Armillaria root rot on highbush blueberry (*Vaccinium corymbosum* L.) in North-eastern Italy (Trentino region)

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Abstract: Since 2003, highbush blueberry plants with abnormal growth characteristics have been found in the Trentino region (North-eastern Italy). The plants are stunted, develop small leaves that redden prematurely in autumn, the roots rot, several branches wilt and usually die within a few months. The microorganisms found on the rotted roots of symptomatic plants were preliminary identified as *Armillaria* sp. In Europe, seven *Armillaria* species have been identified that are usually pathogens of the stressed trees, but that can also survive as saprophytes on wood and root debris. Therefore organic mulch could be a potential source of inoculum of the disease. As the first suspected agent is *Armillaria* sp., we are determining its presence, both on blueberry roots and on the coniferous bark used as cover mulches, and we are evaluating the disease development in commercial plantations.

Armillaria sp. was isolated from roots of symptomatic plants and from bark used for mulching, and then inoculated onto healthy plants to prove the pathogenicity of the isolates to verify Koch-postulate. Molecular genetics methods were used to identify the *Armillaria* species present on infected plants and on nearby mulching bark. The analyses were carried on DNA extracted directly from *Armillaria* mycelium derived from root and bark samples. A blueberry infected plantation was monitored twice per year to observe how the disease spreads and develops. The isolates from the infected plants and bark were both able to produce symptoms on newly inoculated plants and they were identified as *A. gallica* and *A. mellea* by molecular genetic analysis. In the studied plantation, from 2003 and 2005 the disease spread slowly, with an average yearly increase of dead plants that varied between 1% and 2.5%.

Key words: *Armillaria* sp., highbush blueberry, mulching barks

Introduction

In the Trentino region (North-eastern Italy), highbush blueberry (*Vaccinium corymbosum* L.) is a recently introduced crop. In this area, after 15 years of cultivation, in 2004 the cropped surface was 40 ha with a yearly production of 350 tons. Blueberries have a shallow root system and should be mulched with a 10 cm deep layer of organic mulch consisting in bark, sawdust or leaves. Mulch has several positive effects: it increases organic matter, holds moisture in the soil, protects roots from heat and helps to control weeds.

Starting in 2003, in some orchards, stunted plants were found in the region. The plants developed small leaves that reddened prematurely in autumn, the roots rotted, several branches wilted and the symptomatic plant usually died within a few months. The microorganisms found on rotted roots of symptomatic plants were preliminary identified as *Armillaria* sp. They produced white mycelium between the bark and the hardwood and abundant rhizomorphs around the roots and the crown. *Armillaria* species induce root disease on a broad range of plants and cause economical losses, especially on fruit and forest trees (Lochman *et al.* 2004). In Europe, seven biological species have been identified: *Armillaria*

borealis, *A. ectypa*, *A. gallica*, *A. tabescens*, *A. mellea*, *A. ostoyae* and *A. cepistepes* (Guillaumin *et al.* 1985, Shaw & Kile 1991). They are usually pathogens of stressed trees, but they can also survive as saprophytes on wood and root debris (Lochman *et al.* 2004). Therefore organic mulch could be a potential inoculum source of the disease.

The objectives of this work were to identify the causal agent of this blueberry disease, to verify if *Armillaria* sp. is present both on blueberries roots and on the coniferous bark used for mulching and to evaluate the disease development in a commercial orchard.

Material and methods

A blueberry plantation with the typical symptoms of stunted plants and root rot was monitored twice a year (in spring and autumn), starting from November 2003, to observe how the disease spreads and develops. The dead and stunted plants were uprooted and used for the isolation of the putative pathogen. Samples from the different bark types used as mulch in the orchard and samples from symptomatic blueberry plants were kept in the dark, in high relative humidity in plastic bags for a month, to promote mycelium growth of potential pathogens. *Armillaria* rhizomorphs growing on roots of symptomatic plants and mulching bark samples were isolated on malt extract agar medium.

Pathogenicity was tested on blueberry plants: autoclaved apple wood pieces were inoculated with two isolates, one from bark one from roots, on malt extract agar medium in Petri dishes. When the wood pieces were completely colonized, they were placed between roots of two-year old potted blueberry plants, which were then kept in a glasshouse (Prodorutti *et al.* 2005).

Table 1. Samples used for molecular genetics analysis. B, C, G, O, M: DNA samples from *Armillaria* species (two strains of each) used as references. Other samples: DNA extracted from *Armillaria* mycelium derived from root and barks grown on different substrates and extracted directly from rhizomorphs.

CODE SAMPLE	DESCRIPTION
B	*A. borealis* (SSI 522 161 and Lothar 8-11-4)
C	*A. cepistipes* (LWF-LAU 1-1 and SSI 498 145/1-1)
G	*A. gallica* (PBMD4389 and PBMD 6364-1)
O	*A ostoyae* (PBMD 4723 and PBMD 3933)
M	*A mellea* (Brissago and Pbmd 6366-1)
A mir A/B	Mycelium from roots grown on MEA, field 1
A cor A/B	Mycelium from bark grown on MEA, field 1
T A/B	soil near infected plant, field 1
AC	Mycelium from bark grown on Malt broth, field 1
AM	Mycelium from roots grown on Malt broth, field 1
P6Cr	Mycelium from roots plant 6, field 1
P7Cr	Mycelium from roots plant 7, field 1
P1S	Mycelium from roots plant 1, field 2
P10Cr	Mycelium from roots plant 10, field 1
C10C	Mycelium from bark near plant 10, field 1
P1Cr	Mycelium from roots plant 1, field 1
P2Cr	Mycelium from roots plant 2, field 1

The identification of *Armillaria* species present on infected plants and on nearby mulching bark was done using a PCR-based method. The experiment was carried on DNA extracted from *Armillaria* mycelium derived from root and barks samples grown on different substrates (Table 1) and extracted directly from rhizomorphs, using NucleoSpin Plant® DNA extraction kit (Macherey-Nagel). Ten DNA samples derived from five *Armillaria* species (two strains each one) were used as references for the different species (Table 1). In the PCR analysis, seven primer combinations designed on internal transcribed spacer (ITS) and intergenic spacer (IGS) regions of rDNA were used (Lochman *et al.* 2004, Pérez-Sierra *et al.* 2000, Sicoli *et al.* 2003) and the amplicons were separated on agarose gels (Table 2). To confirm the bands identity, restriction fragment length polymorphisms (RFLPs) analysis with restriction enzyme (AluI) was done on amplicons obtained by amplification with LR12R/O1 primer combination (Pérez-Sierra *et al.* 2000).

Table 2. Specific primers designed on ITS and IGS regions of rDNA, used for amplification of *Armillaria* DNA.

Primer	Sequence (5'→3')	Lenght	Amplicons	Reference
ITS1	TCCGTAGGTGAACCTGCGG	20	847-882	Lochman J. *et al.*,
ITS4	TCCTCCGCTTATTGATATGC	20		2004
AR1	CTGACCTGTTAAAGGGTATGTGC	23	690-724	Lochman J. *et al.*,
AR2	AAGCTGAATCCTTCTACAAAGTCAA	25		2004
ATA1	TTGCCTTGAACCCTGTTATAAGGC	24	329-335	Sicoli G. *et al.*,
ATA2	TGCCAAAATCGTTGCACGCCGC	22		2003
AMEL3	TTGCTTGCTTACGAGCTAAG	20	517	Sicoli G. *et al.*,
ITS4	TCCTCCGCTTATTGATATGC	20		2003
AME1	AAGAATCATGAGATATCATCAGT	23	319-342	Sicoli G. *et al.*,
AME2	TTAGAAAATCCGCCTTAGAAAC	22		2003
AOS1	CAGRTAAAGCTAACAACAACTTT	23	314-317	Sicoli G. *et al.*,
AOS2	AAARTTTGAACGTAGCCCTARA	22		2003
LR12R	CTGAACGCCTCTAAGTCAGAA	21	845-920	Harrington T.C *et*
O-1	AGTCCTATGGCCGTGGAT	18		*al.*, 1995

Results and discussion

The isolates from the infected plants and from the different bark used as mulch were identified as *Armillaria gallica* and *A. mellea* by RFLPs analysis. Two isolates from bark and six from roots, derived from the first studied plantation had an identical pattern to one of the references for *A. gallica* (Fig. 1 and 2). In another infected field we have identified *A. mellea* on roots of a stunted plant; DNA was extracted directly from mycelium and one isolate had the typical pattern for *A. mellea* (Fig. 2).

The isolates from the infected plants and from the bark were both able to produce symptoms on newly inoculated plants: ten months after the inoculation with the isolates, the blueberry plants showed similar symptoms as detected on symptomatic plants in the original plantation of isolation.

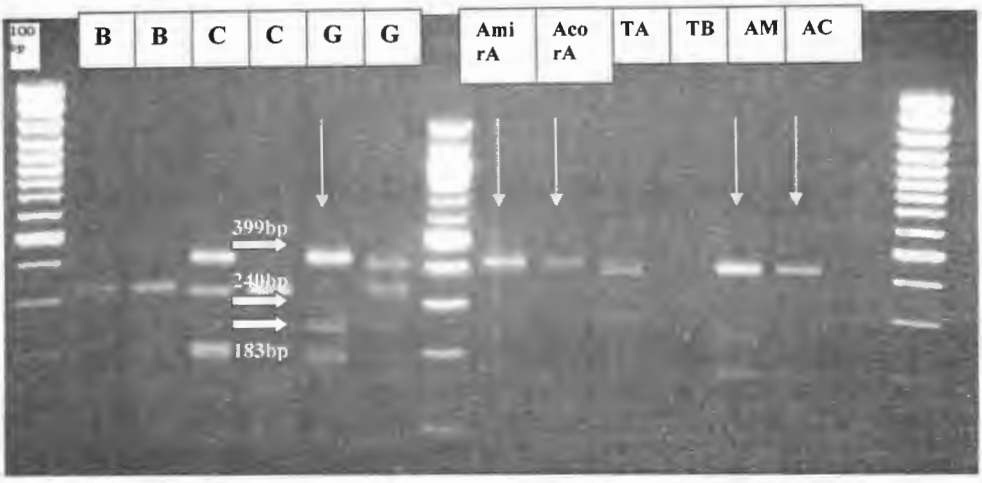

Figure 1. RFLPs profile restriction with the enzyme AluI. Lanes 2 and 3, *A. borealis* (SSI 522 161 and Lothar 8-11-4); Lanes 4 and 5, *A. cepistipes* (LWF-LAU 1-1 and SSI 498 145/1-1); Lanes 6 and 7, *A. gallica* (PBMD4389 and PBMD 6364-1); Lanes 9 and 10, DNA extracted from blueberry and bark mycelium grown on MEA; Lanes 11 and 12, DNA extracted directly from soil; Lanes 13 and 14, DNA extracted from blueberry and bark mycelium grown on MALT broth. The arrows indicate the isolates with the same pattern as the first reference strain for *A. gallica* and the molecular weight (base pair) of the fragments obtained.

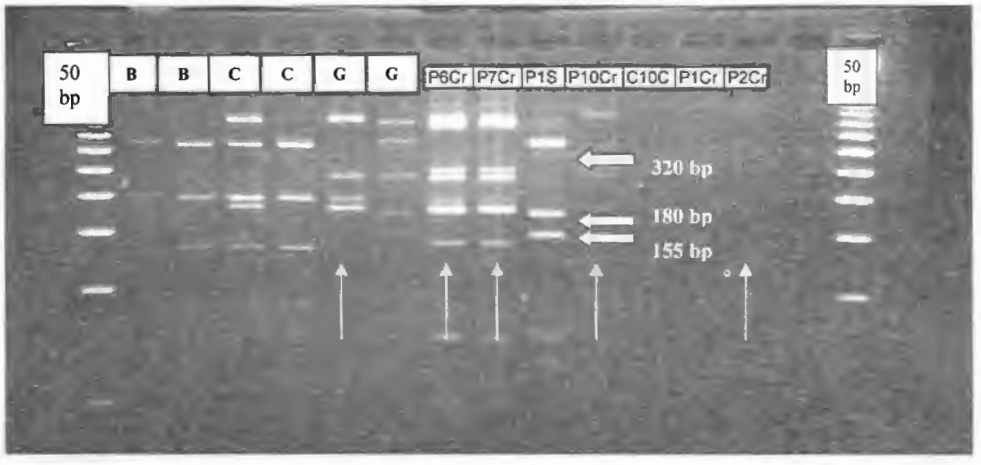

Figure 2. RFLPs profile restriction with the enzyme AluI. Lanes 2-3, *A. borealis*; Lanes 4-5, *A. cepistipes*; Lanes 6-7, *A. gallica*; Lanes 8-9, mycelium from roots plant 6 and 7, field 1; Lane 10, mycelium from roots plant 1, field 2; lane 11-12, mycelium from roots plant 10 and mycelium from bark near plant 10, field 1; Lane 13-14, mycelium from roots plant 1 and plant 2, field 1. The vertical arrows indicate the isolates with the same pattern as the first reference strain for *A. gallica.* The horizontal arrows indicate the pattern for *A. mellea* with the molecular weight (base pair) of the fragments obtained.

In the period from 2003 to 2005 the disease was slowly spreading in the studied plantation, with an average seasonal increase of dead plants that varied between 1% (from May to October 2004) and 2.5% (from October 2004 to May 2005). During 2005 the number of dead plants was stable while the stunted plants increased (Table 3). In November 2003, the number of stunted and dead plants was bigger than in May 2004: some plants seemed dead in the autumn but in the spring they produced new shoots. Probably this depended on the drought year in 2003 (Table 3).

Table 3. Monitoring of blueberry orchard: number of healthy, stunted and dead plants.

Date/Number of plants	healthy plants	stunted plants	dead plants
November 2003	469	61	17
May 2004	506	28	13
October 2004	475	45	27
May 2005	455	60	32
October 2005	450	65	32

Date/Percentage	healthy plants	stunted plants	dead plants
November 2003	85.7	11.2	3.1
May 2004	92.5	5.1	2.4
October 2004	86.8	8.2	4.9
May 2005	83.2	11.0	5.9
October 2005	82.3	11.9	5.9

A. gallica and *A. mellea* should be considered the causal agent of the highbush-blueberry dieback detected in Trentino Region (Italy).

A. mellea and *A. ostoyae* have been reported on highbush blueberry in the USA (Caruso 1995) but to our knowledge this is the first report of root rot caused by *A. gallica* on this crop (Prodorutti *et al.* 2005).

Since *A. gallica* and *A. mellea* were found on the mulch and, as pathogens, on the plant, the different bark used could be a source of inoculum in the field, promote the spread of the disease or simply represent an alternative substrate colonised by the pathogen after plant infection. This is why our future work will focus on a large-scale analysis on blueberry plants from different infected orchards in Trentino, to confirm the species identity and to verify the role of bark as a source of inoculum.

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Diseases of high-bush blueberry in Integrated Production plantations in Slovenia

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Abstract: Mummy berry disease and ripe fruit rot are the most important fungal diseases of high-bush blueberry in Slovenia. Mummy berry disease is caused by *Monilinia vaccinii-corymbosi,* a major pathogen of *Vaccinium corymbosum* throughout North America, which has recently also been found in Europe. Ripe fruit rot is caused by *Colletotrichum acutatum* sensu lato. Isolates of both pathogens were identified and characterised based on morphology and sequence data of the internal transcribed spacer region of ribosomal DNA and the β-tubulin gene.

Key words: *Vaccinium corymbosum, Monilinia vaccinii-corymbosi, Colletotrichum acutatum*

Introduction

The diseases that affect high-bush blueberry production in Slovenia most severely are mummy berry disease caused by *Monilinia vaccinii-corymbosi* and ripe fruit rot caused by *Colletotrichum acutatum.*

The mummy berry disease caused by *M. vaccinii-corymbosi* is a major disease of *Vaccinium corymbosum* and related *Vaccinium* species throughout North America. It causes two kinds of damage: blighting of the emerging leaves, shoots and flower clusters and mummification of maturing fruits.

M. vaccinii-corymbosi and other species of *Monilinia* specifically pathogenic to ericaceous hosts, belong to *Disjunctoriae* group (Batra 1991). These species form a sexual and an asexual reproductive stage. In spring, stalked brownish apothecia develop on stromatized fruits. These are also called pseudosclerotia. Ascospores cause primary infections of young leaves and shoots. Conidia are then produced on the blighted plant parts and further infect flowers, from which mummified fruits develop.

M. vaccinii-corymbosi was first documented in Europe in 2003 in Austria (Gosch 2003). A year later (summer 2004) blueberry growers in Slovenia found the first infected and mummified fruits in their plantations. In spring 2005, severe infection of new shoots and flowers occurred. Two flushes of blight were observed following two periods of cold and wet weather. At maturity, infected fruits mummified and fell to the ground. *Vaccinium corymbosum* 'Berkeley', 'Coville' and 'Rancocas' were the most affected varieties where the extent of damage reached 70 - 100 %. The outbreak in 2005 was most probably related to cold weather in early spring since damage caused by frost was observed on the truss buds of several cultivars. It is known that frost damage is an important predisposing factor for infection by *M. vaccinii-corymbosi* and condition a higher incidence of mummy berry disease (Hildebrand & Braun 1991).

The origin of *M. vaccinii-corymbosi* and details about epidemiology of the disease in our production system are not clear as yet. It is possible that the fungus was introduced with planting material obtained from outside Europe and remained undetected so far. Some blueberry growers claim to have seen monilinia-like symptoms now and then during the past ten years, but

their observations were not verified by fungus isolation and identification and could therefore be false assumptions.

Colletotrichum acutatum is another pathogen that is becoming increasingly important in our blueberry production. Damage due to ripe fruit rot has frequently been reported in the last years, particularly for the variety 'Coville'. *C. acutatum* was first recorded in Slovenia in 1999, when it caused severe outbreaks of fruit and crown rot in strawberry plantations.

C. acutatum is considered to be a species complex. Several subspecific groups were described on the basis of morphology, RAPD analysis, mt DNA RFLPs and DNA sequences of several genes. Our aims were to identity and classify strains isolated from blueberry and strawberry into six molecular groups (A1 - A6) according to Talhinhas et al. (2005).

Material and methods

Fungal isolates

Fourteen isolates of *M. vaccinii-corymbosi* and *C. acutatum* were examined in this study. Strains of *M. vaccinii-corymbosi* (M 311, M 312) were obtained from high-bush blueberry plantations in Slovenia and Austria. They were isolated from macroconidia that developed on blighted twigs and flower clusters and identified by colony characteristics, morphology of hyphae, macroconidia and microconidia, presence of disjunctors as well as characteristics of pseudosclerotia and apothecia. Strains of *C. acutatum* (c102 – c104) originated from two high-bush blueberry plantations in Slovenia. They were isolated from blighted leaves, twigs and rotten ripe blueberry fruits. Cultural features (colour of aerial mycelium, pigments in agar, radial mycelial growth rate in the dark at 20 °C) as well as morphological features (conidial shape and size) were used for their identification. Nine strains of *C. acutatum* from *Fragaria x ananassa* (c075 - c096) were also included in the study. They were collected during surveys in the years 1999 - 2001 and isolated from spore masses that developed on strawberry petioles after treatment with paraquat.

Phylogenetic analysis

The mycelium of the strains studied was grown in liquid medium. DNA was extracted using the Qiagene DNeasy Plant Mini Kit. Primers ITS1/ITS4 (White et al. 1990) and T1/T22 (O'Donnell & Cigelnik 1997) were used in amplifications. The ITS1, 5.8S rDNA, ITS2 of the rDNA cluster of *Monilinia* strains was amplified using the following program: 180 sec at 94 ^{0}C for initial denaturing; 35 cycles of 40 sec at 94 ^{0}C (denaturing), 50 sec at 54 ^{0}C (annealing), 120 sec at 72 ^{0}C (prolongation); followed by a final prolongation step at 72 ^{0}C. A similar program was used for the partial β-tubulin gene of *Colletotrichum* strains. To avoid amplifications of pseudogenes, an annealing temperature of 58 ^{0}C was used in the first 8 cycles of the PCR followed by 32 cycles using an annealing temperature of 52 ^{0}C. In sequence reaction, the primers ITS4 for the rDNA and T2 (O'Donnell & Cigelnik 1997) for the partial β-tubulin gene were used. Post PCR treatment included purification of PCR products, sequence reactions using BigDyeTM (Applied Biosystems) terminator cycling conditions, purifying sequence reactions by ethanol precipitation and analyzing the sequences on an automated 3730xl sequencer (Applied Biosystems). Quality checked sequences were aligned using the software package Mega3 (Kumar et al. 2004) together with sequences downloaded from public databases. Sequences of *Monilinia* and *Colletotrichum* species, mainly from studies of Holst-Jensen et al. (1997), Ioos & Frey (2000), Sholberg et al. (2003) and Talhinhas et al. (2002, 2005) were downloaded. The similarity of sequences of strains included in these studies was analyzed using the Neighbour-Joining algorithm as implemented in Kumar et al. (2004).

Results and discussion

Monilinia vaccinii-corymbosi

The main morphological and cultural characteristics of the studied strains corresponded with characteristics described by Batra (1983). Pseudosclerotia, which developed within mummified fruits, were hollow, ribbed, with flattened ends. In spring they gave rise to brownish, cup-shaped apothecia (10 - 20 mm in diameter) with long stipes. Colonies grew slowly and reached 9 cm in diameter after 21 days of growth on PDA at 20 °C in the dark. Mycelium was white to beige and compact. Brown stroma developed in older cultures. Reverse of plates was brown, with yellow crystals visible in some cultures. Hyphae were broad and often assembled in fascicles. Macroconidia were formed in long chains with 2 - 3 µm long disjunctors separating individual conidia. They were limoniform, hyaline, smooth, and measured 16.4 – 22.6 x 11.2 – 17.1 µm (in culture). Production of macroconidia was abundant on blighted shoots and flowers but scarce in pure culture. Microconidia were globose, 3 - 4 µm in diameter, abundantly produced on hyphae.

Phylogenetic analysis revealed that strains from Slovenia and Austria belong to the group of *Monilinia* species specifically pathogenic to *Ericaceae* with fleshy berries: *M. urnula, M. baccarum, M. megalospora, M. oxycocci, M. polycodii, M. vaccinii-corymbosi* and *M. gaylussaciae* (Figure 1). They were closely related but not identical with North American isolates of *M. vaccinii-corymbosi* and *M. gaylussaciae*. Further comparison of isolates of *M. vaccinii-corymbosi* from populations in North America and Europe is necessary to obtain more information about identity and origin of European isolates.

Colletotrichum acutatum

Size and shape of conidia of *C. acutatum* strains isolated from blueberry were very variable. Conidia were cylindrical to fusiform, rounded or pointed at one or both ends and measured 8,8 – 16 x 3.2 – 4,7 µm. Colonies were initially white, later grey with white margin and highly chromogenic – excreting carmine red pigment into the culture media. Average colony diameter after 7 day growth on PDA at 20°C was 53 mm.

Dendrogram of *Colletotrichum* strains (Figure 2) shows that isolates of *C. acutatum* studied here belong to three different molecular groups (A2, A3, A4) of Talhinhas et al. (2005). All isolates originating from *Vaccinium corymbosum* and isolate c088 from *Fragaria x ananassa* clustered in molecular group A3. These isolates are characterised by strong production of carmine pigments, which is not typical for other representatives of this molecular group, isolated from *Olea europea* and different ornamentals. In the study of Talhinhas et al (2005) all chromogenic isolates grouped in molecular group A5 which included also *C. acutatum* type specimens. None of our isolates was placed in this group.

The majority of isolates from *Fragaria* x *ananassa* grouped in molecular group A2. They had uniform, whitish to grey colonies, pink on reverse. They were mostly isolated from planting material, tested for latent infection with *C. acutatum*. Isolate c088, placed in molecular group A3, was distinct from all other strawberry anthracnose isolates and originated from a garden where no planting material has been introduced for several years.

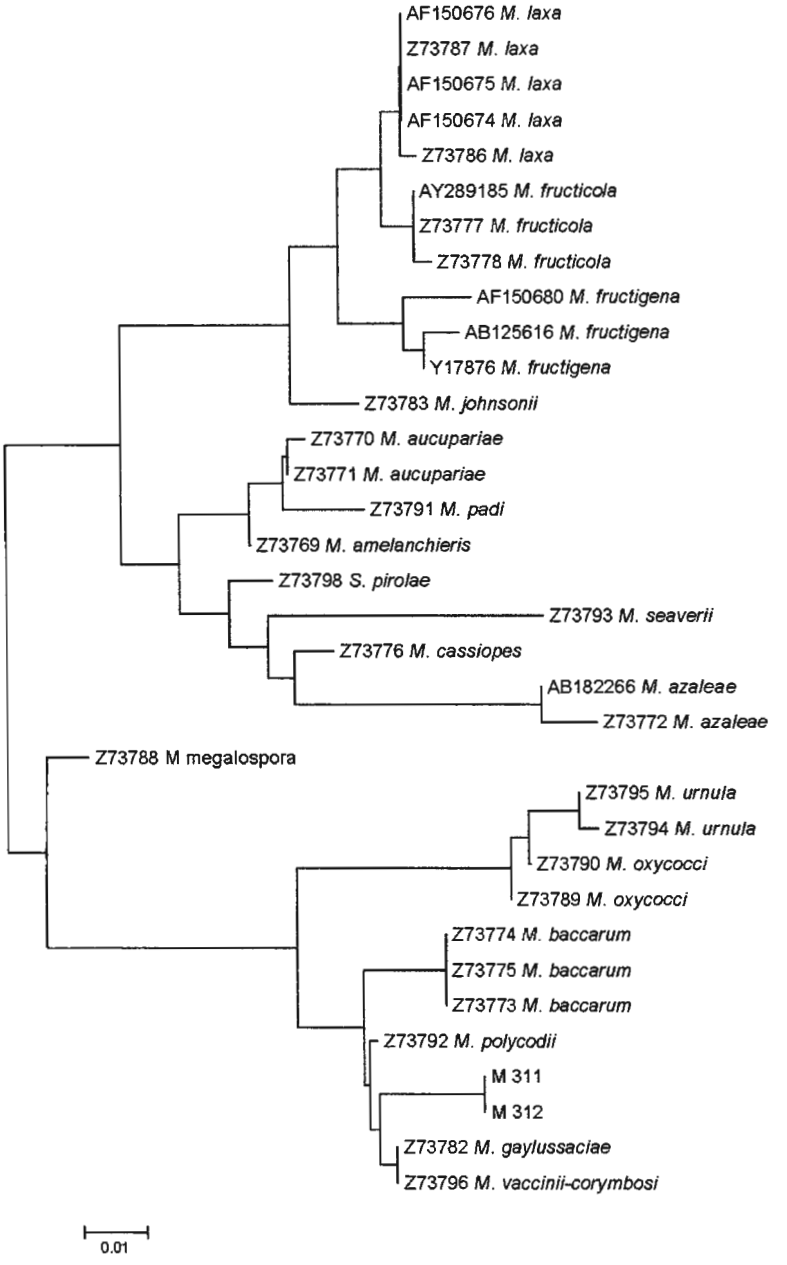

Figure 1. Dendrogram obtained from a neighbour-joining analysis of ITS sequence data of *Monilinia* species. M 311 – isolate from Slovenia, M 312 – isolate from Austria, other sequences obtained from studies of Holst-Jensen et al. (1997), Ioos & Frey (2000), Sholberg et al. (2003).

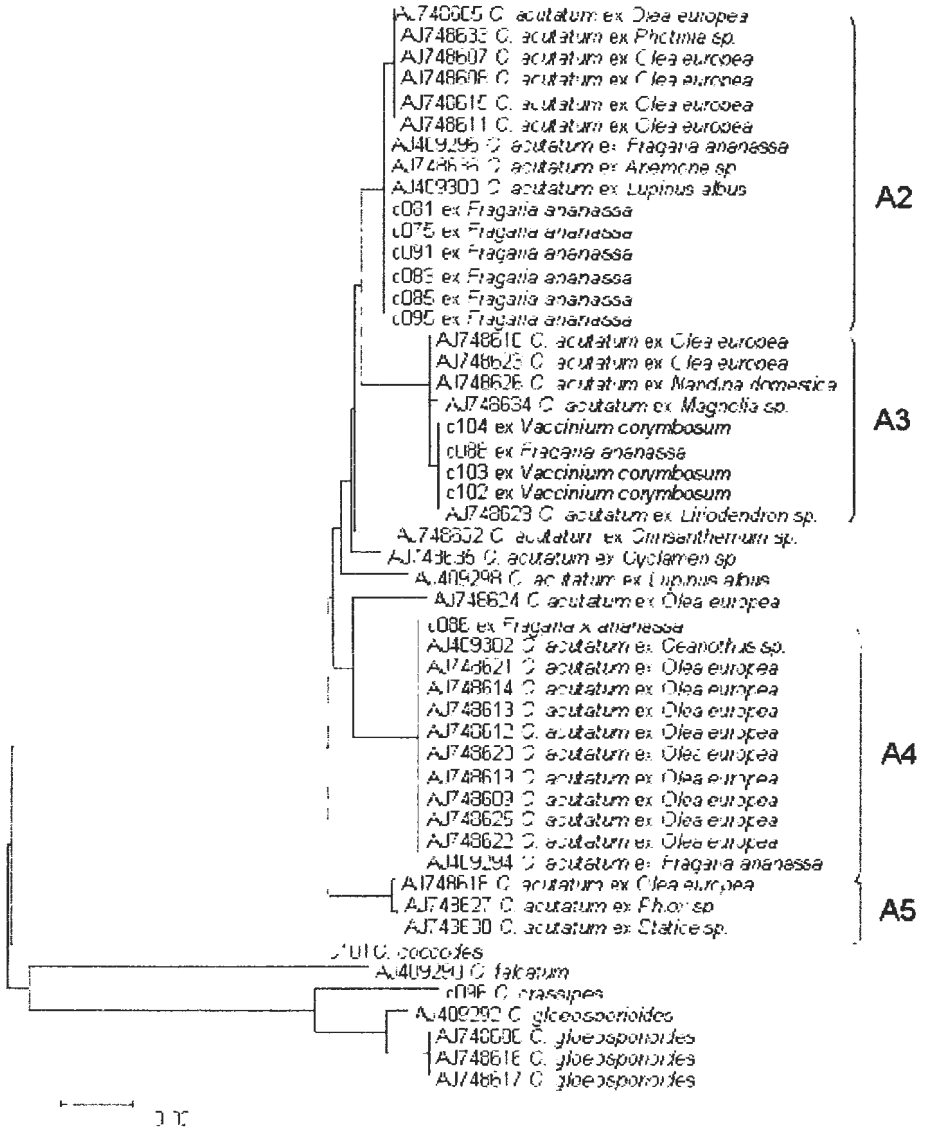

Figure 2. Dendrogram of *Colletotrichum* isolates obtained from a neighbour-joining analysis of β-tubulin sequence data. Sequences of reference isolates were obtained from studies of Talhinhas et al. (2002, 2005). A2 - A5: *C. acutatum* molecular groups (Talhinhas et al. 2005).

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Colletotrichum acutatum in Norwegian strawberry production and sources of potential inoculum in and around strawberry fields

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Abstract: *Colletotrichum acutatum*, the cause of strawberry blackspot, was detected on strawberry (*Fragaria* × *ananassa*) for the first time in 1999 in Norway. Since then, the fungus has been detected in 12 nurseries producing strawberry plant material (at the elite plant station, and in 8 certified and 3 non-certified nurseries), in 17 strawberry fields and in a strawberry planting that was part of a breeding programme. No symptoms of the disease were ever detected on plant material included in the testing, and the fungus was only discovered after using the paraquat test on petioles from strawberry leaves. Only a few times, symptoms have developed on strawberry fruits. In 2002 and 2003, the disease was found as latent infections on several cultivars in the nuclear plant collection at the national elite plant station. None of the ornamental plants (81 different species) grown in the same greenhouse complex as the elite plants tested positive for *C. acutatum*. In 2004 and 2005, we tested plant material from vegetation in and around infested strawberry fields. *C. acutatum* was detected on two *Cotoneaster* species, sour cherry (*Prunus cerasus*), blackberry (*Rubus fruticosus*), raspberry (*R. idaeus*), and the weeds northern dock (*Rumex longifolius*) and common nettle (*Urtica dioica*). The fungus is common on all fruit crops grown in Norway (apple, pear, plum, sweet- and sour cherry), and recently we have also found it on highbush blueberry (*Vaccinium corymbosum*) and holly (*Ilex aquifolium*). Genetic variation between isolates of *C. acutatum* from strawberry and all tree fruit species was studied by means of AFLP (amplified fragment length polymorphism). Preliminary results showed that the isolates were not clearly grouped according to host origin, and there were examples of complete match between isolates from various hosts, including strawberry.

Key words: AFLP, blackspot, *Fragaria* × *ananassa*, fruits, ornamentals, weeds

Introduction

Blackspot in strawberry (*Fragaria* × *ananassa*), caused by *Colletotrichum acutatum*, is a relatively new disease to the strawberry production in Norway and elsewhere in Europe. The fungus may attack the whole plant, but typical symptoms are most apparent on fruits. Infected fruits develop brown circular, sunken lesions that contain orange, moist spore masses, and eventually the whole fruit will shrivel and become dark. Complete destruction of marketable yields has been reported from strawberry fields in England (Berrie & Burgess 2003) and Denmark (Bisgaard 2002). The first outbreak of strawberry blackspot in Norway was in 1999, when it was detected in greenhouse-grown strawberries (Stensvand et al. 2001).

C. acutatum is a major disease causing fungus in several important crops worldwide, and has a wide host range (Peres et al. 2005, Smith et al. 1997). In Norway, *C. acutatum* has caused disease (anthracnose) on tree fruit crops (apple, pear, plum, sweet- and sour cherry) grown in the country, and it is especially severe in sweet- and sour cherry and in some apple

cultivars. There have only been a few instances of blackspot on strawberry fruits in Norway, but an increasing number of latent infections have been detected on stipules/petioles sampled from field production of runner plants (Sletten et al. 2005).

The first attempts to establish testing of plant material for presence of *C. acutatum* started in 2001, and regular testing of certified strawberry plant material has been carried out since 2003. When the testing was initiated, the fungus was detected both in nuclear, elite and certified production plants and caused a serious setback of the entire Norwegian certified production (Sletten et al. 2005). The elite plant production was stopped for one year, and in more than half of the certified nurseries latent infections of *C. acutatum* were discovered. Because of its damaging potential to the strawberry industry, *C. acutatum* in strawberry is considered a quarantine organism to Norway, and also to EU. The disease must be notified, and strict measures may be set in force to eradicate outbreaks. There are no quarantine regulations for *C. acutatum* in other crops.

Weeds and other vegetation in and around strawberry fields may host *C. acutatum* (Berrie & Burgess 2003; Freeman et al. 2001a, MacKenzie et al. 2004). Thus, the pathogen may spread from strawberry to weeds adjacent to strawberry fields and then reinfest new strawberry fields the following year. Molecular and vegetative compatibility studies indicated that isolates from weeds, anemone and strawberry were from a single population in Israel (Freeman et al. 2001b).

In this paper we give an overview of the presence of *C. acutatum* in Norwegian certified production of strawberry runners, in regular strawberry production, and in vegetation in and around strawberry fields known to host the fungus. Furthermore, we report from initial work on the genetic diversity of isolates of *C. acutatum* from strawberry and fruit crops.

Materials and methods

There is one elite plant station in Norway, located at Sauherad in Telemark county. It has a collection of nuclear plants and produces elite plants for certified growers and other plant producers. In 2003, there were 17 producers of certified runner plants. In 2004 and 2005, there were only 7 producers left. Samples were collected from nuclear, elite and certified plants in 2001 to 2005 (the latter only from 2003) by regional inspectors of the Norwegian Agricultural Inspection Service and investigated further at The Norwegian Crop Research Institute (NCRI; from 2006 Bioforsk), procedures described by Cook (1993) and recommended by EPPO (2004) were followed. For elite and certified plants, the stipule and 3 cm of the petioles of the oldest green leaf on each of 300 plants were included in each sample (one cultivar in one field). In some certified fields containing a limited number of plants from certain minor cultivars, more than one cultivar was included in the 300 plants. There were few nuclear plants of each cultivar, and only 17 to 40 stipules/petioles were included in each sample (material from all plants for each cultivar was included). The stipules/petioles were treated with paraquat according to the procedure developed by Cook (1993). After six days incubation (100% RH, continuous light and 25°C) the stipules/petioles were examined for presence of *C. acutatum* in a dissecting microscope. Presence of the fungus was confirmed by microscopy. The fungus was isolated, and species identification was confirmed by PCR (Martínez-Culebras et al., 2003; Sreenivasaprasad et al., 1996).

At the elite plant station there was a large stock of ornamental plants, both perennials and woody ornamentals. Eighty one different species (including one to many cultivars of each species) were tested for presence of latent infection of *C. acutatum*. The paraquat test was carried out on a limited number of plants of each cultivar/species, including one or more of the older leaves on a plant as for strawberry described above.

Plant material from regular strawberry production was also tested for presence of *C. acutatum*. Since 2003, the diagnostic clinic at NCRI has received plant material from strawberry growers to test for *C. acutatum*. Furthermore, in 2003 and 2004, plants from a total of 73 strawberry growers were investigated for presence of *C. acutatum* following the procedures described above.

In 2004 and 2005, we investigated vegetation in and around strawberry fields where *C. acutatum* had been confirmed present in strawberry plantings. The vegetation included weeds, fruit- and berry crops and ornamentals. Six strawberry fields and 77 different plant species were included in 2004, and the following year, ten fields and 114 different plant species were included. Leaves, petioles, stipules, stems, and occasionally fruits and nuts were included in the paraquat tests, and number of plants and plant parts varied for each species.

Genetic variation was studied by means of AFLP (amplified fragment length polymorphism) with isolates of *C. acutatum* from strawberry and tree fruit crops. The numbers of isolates from Norway included in the test were: 24 from strawberry, 13 from sweet cherry, 21 from sour cherry, 5 from plum, 16 from apple and 1 from pear. In addition, one isolate of *C. acutatum* from citrus (provided by Central Science Laboratory, York, England) and four isolates of *C. gloeosporioides* (3 from USA and 1 from England) were included in the test.

Results and discussion

In 2001 to 2003, several cultivars of the nuclear stock at the elite plant station were found to be infected by *C. acutatum*. In 2003, the fungus was also detected on elite plants and on plants from seven certified and three non-certified plant producers. The following year, *C. acutatum* was only detected in one certified nursery. Due to the severe situation that occurred at the elite plant station, a complete renovation of the nuclear stock and elite plant production was carried out in 2003/2004, and in 2005 we have not been able to detect the fungus in any part of the certified strawberry plant production.

We did not find *C. acutatum* in any of the ornamental plants at the elite plant station. However, in a virus-free stock of sweet cherry trees (used as a scion bank) adjacent to the greenhouses used for the nuclear and elite strawberry plants, we found both buds and leaves infected by *C. acutatum* (J. Børve & A. Stensvand, unpublished data). Thus, even if movement in and out of the greenhouses used for strawberry plant production is very restricted, the sweet cherry trees may have been a potential source of inoculum for the strawberry plants.

C. acutatum was detected (asymptomatically on stipules/petioles) in 13 fields for regular strawberry production in 2003-2004, and over the same period the diagnostic clinic received four strawberry fruit samples containing the fungus (from growers). The fungus was also detected in a strawberry planting that was part of a breeding programme.

From vegetation in and around infected strawberry fields, the fungus was found on two *Cotoneaster* species (*C. bullatus* and *C. lucidus*), *Prunus cerasus* (sour cherry), *Rubus fruticosus* (blackberry), *Rubus idaeus* (raspberry), *Rumex longifolius* (northern dock) and *Urtica dioica* (common nettle) in 2004. In 2005, *C. acutatum* was detected on raspberry adjacent to two different strawberry fields. Recently (not part of this testing), we have also found the fungus on *Vaccinium corymbosum* (highbush blueberry) and *Ilex aquifolium* (holly).

The results from the AFLP test are only preliminary, but all the *C. acutatum* isolates were clearly different from *C. gloeosporioides*. The Norwegian isolates were not clearly grouped according to host origin. Examples of 100% similarity obtained between isolates

from various hosts were: (i) isolates from plum and apple from Hordaland county, strawberry from Rogaland county and sour cherry from Buskerud county, (ii) isolates from sour cherry, sweet cherry and plum from Hordaland county and strawberry from Aust-Agder county, (iii) one strawberry isolate from the elite plant station and four isolates from certified strawberry producers. The latter group with 100% similarity indicates that the fungus was spread from the elite plant station to the certified producers.

From the present work we can conclude that *C. acutatum* has many host plants in Norway, and besides strawberries, different fruits, and highbush blueberries, it may be found resident in weeds and ornamental plants. Furthermore, there may be close genetical similarities between isolates from different hosts (including strawberry). The wide host range of *C. acutatum* confirms what is found in many other countries (Peres et al., 2005; Smith et al., 1997). In the future, we will run more AFLP analyses that will include isolates from weeds and other vegetation and investigate possible cross infection between different host plants under natural conditions. This information will be of particular importance for producers of certified strawberry plants and the plant health authorities when approving certified fields.

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Detection of *Colletotrichum acutatum* and Black currant reversion virus (BRV) from planting material of strawberry and currants

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Abstract: *Colletotrichum acutatum*, a quarantine organism on strawberries in the EU, was found in Finland for the first time in 2000 in imported strawberry plants. Concern about rapid, unnoticeable spread of this pathogen has necessitated studies to find methods to detect the quiescent fungal infection in imported, cold-stored strawberry plant material.

Successful detection of *C. acutatum* in strawberry tissues by polymerase chain reaction (PCR) is dependent on the method of DNA extraction used. High-quality nucleic acid, free from PCR inhibitors, was successfully prepared by slightly modifying the DNA extraction method of a commercially available kit. Species-specific primers were successfully used in the PCR reaction. *C. acutatum* was detected by PCR both on symptomatic and asymptomatic plant parts such as runners, petioles and crowns of naturally and artificially infected strawberry plants. The results show that the PCR technique can be used to detect *C. acutatum* in strawberry tissue even in plant parts that do not show any visible symptoms. The Plant Quarantine Laboratory in Finland has used the bioamplification ELISA to test strawberry planting material. In 2005, PCR has been used also in preliminary testing in addition to the official ELISA test.

Black currant reversion-associated virus was originally isolated in Finland from reverted black currant in 1990. The virus was found to be the causal agent of reversion disease and was named *Black currant reversion virus* (BRV). The virus also infects other *Ribes* species. The first assay, IC-RT-PCR, for the detection of BRV was based on the combination of virus capture using antibodies with moderate specificity and nucleic acid-specific detection. RNA purification kits without the use of antibodies have also been successfully used to detect BRV in black currant samples. BRV can be detected from leaves, galls and gall mites (*Cecidophyopsis* spp.) collected from reverted plants. The virus can have very uneven distribution in plants, and sampling is of vital importance in reliable detection. BRV is tested from propagation material of black, red and white currants produced in the Elite Plant Station of Finland with the IC-RT-PCR method.

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Area-wide application of pheromone mediated mating disruption in sustainable IPM

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Abstract: Pheromone mediated mating disruption (MD) is now a major tool of sustainable Integrated Pest Management (IPM) systems in Horticulture. In Australian orchards, MD techniques are used for successful and long-term sustainable control of oriental fruit moth (OFM), codling (CM) and light brown apple moth (LBAM). Initially the general approach was to treat individual orchard blocks and only known hosts with MD. The incidence of OFM damage on the borders of MD treated blocks stimulated an area-wide application of MD for better crop protection. An area-wide MD program, with more than 1,100 ha of 40 contiguous orchards covered with MD dispensers, applied to all fruit trees in the Cobram region of northern Victoria, Australia, substantially improved protection against OFM damage. Detailed monitoring of pest population and shoot tip and fruit damage assessments indicated that application of area-wide MD during two consecutive seasons provided sufficient OFM control. The area-wide MD in the first year helped growers to reduce the number of insecticide sprays by half and in the second year, most of the growers did not spray against OFM at all. Hot spots and edge infestations of OFM were reduced or eliminated through use of the area-wide approach. Later, local growers took the initiative to continue this area-wide MD program as a self-sufficient community approach and achieved the re-establishment of MD across the whole Cobram region. Although successful, the area-wide MD program was an expensive approach. To reduce the cost of area-wide MD program for control of OFM and CM together, trials were conducted where only infested blocks and border areas were treated with MD. Such a selective approach was successful for control of localised pest outbreaks and areas of increased infestation. Pheromone based IPM and selective area-wide MD programs are the key elements in development of cost-effective strategies for pest control, while protecting the environment by reducing pesticide pressure.

Key words: area-wide mating disruption, codling moth, integrated pest management, oriental fruit moth, sex pheromone

Introduction

Oriental fruit moth *Grapholita molesta* Busck (OFM), codling moth *Cydia pomonella* L. (CM) and light brown apple moth *Epiphyas postvittana* Walker (LBAM) (Lepidoptera: Tortricidae) are the main orchard pests in Australia. In Victoria, CM mainly damages pome fruit (mostly apples and pears), while OFM mainly damages stone fruit (mostly peaches and nectarines). LBAM is a native Australian polyphagous pest damaging stone, pome and soft fruit. However, in the last 10 years OFM has become a problem on pome fruit, especially pears in Australia. These pests have the ability to migrate and invade new host-plants and Victorian growers now need to cater for both OFM and CM together in their pest management strategies.

The structure of the female sex pheromone for OFM (George 1965, Carde et al. 1979), CM (Roelofs et al. 1971, Arn et al. 1985) and LBAM (Bellas et al. 1982) has been identified and stimulated investigation of new selective methods for control of these pests. The method when the ability of males to locate virgin females has been restricted by the release of large

amounts of sex pheromone, has been termed mating disruption (MD) (Rothschild 1975). Such MD treatments of orchard blocks could be as effective in OFM control as insecticides (Rothschild 1979). MD may become even more effective, when all orchards in a district are treated to reduce the likelihood of mated OFM females migrating from untreated areas (Vickers et al. 1985).

Experiments with area-wide MD treatments were established for OFM control in South Africa (Barnes and Blomefield 1997) and for CM control in western USA (Calkins 1998). These experiments were successful, but when MD applications were interrupted or stopped, OFM and CM populations quickly increased and caused severe damage again. Such experiments suggested that the success of area-wide MD treatment depended on continuous treatments during a number of consecutive seasons and effective management of borders of MD treated orchards and blocks. For example in Australia, the most severe OFM damage was typically found at the edge of peach blocks under MD, adjacent to pear blocks under insecticide treatments. This pattern of damage has also been reported in USA as a problem in MD treated orchards (Gut and Brunner 1998). Field observations (Il'ichev et al. 1999a) indicated that migration of mated OFM females from pear blocks under insecticide treatment to adjacent peach MD blocks resulted in damage at the edge of the peach MD blocks. Edge damage can quickly spread further to the interior of MD treated fruit blocks. Such outbreaks of higher OFM population levels were recorded in Victoria and were commonly referred to as "hot spots" (Il'ichev et al. 1999b).

An area-wide MD program, in which all fruit varieties were treated with MD during two consecutive seasons in 1997-99, improved the protection of 40 contiguous orchards (1,100 ha) in the Cobram region of Northern Victoria. This area-wide MD program reduced migration of mated OFM females, prevented the edge damage common in smaller orchards treated with MD, and helped many growers to reduce the number of insecticide sprays used against OFM from 14 sprays per season to nil (Il'ichev et. al. 2002).

To maintain the advantage of an area-wide MD over as many years as possible, it is important to look at the most cost effective way of applying MD on pome fruits, as well as to maintain the sustainable MD strategy against OFM and CM in all IPM orchards.

Selective area-wide MD treatments of major pest outbreaks incorporated into pheromone based IPM programs have potential as elements in cost effective control of pests while protecting the environment by reducing the amount of pesticides applied in horticultural crops.

Material and methods

Application of the area-wide MD treatments

To conduct the first area-wide MD experiment (1997-99), the fruit production area located south of Cobram, northern Victoria, was chosen because most of the growers were already using MD treatments in their stone fruit blocks. The most severe OFM damage in the Cobram area was typically found at the edge of peach blocks under MD adjacent to pear blocks under insecticide treatments. The main objective was to investigate whether applying MD to all orchards in an area-wide basis would improve the effectiveness of MD by reducing or preventing the occurrence of OFM hot spots and edge damage.

Cobram growers treated with MD about 550 ha of separated peach and nectarine blocks before the start of an area-wide MD experiment in the 1996-97 season. Blocks of pears, apples, plums and apricots on the same orchards were under an insecticide control program with spray applications of parathion-methyl and/or azinphos-methyl. MD in stone fruits was

usually applied before the end of September and insecticide treatments were applied between 7 and 14 times during the season.

An area-wide MD experiment was established in September-October 1997, in over 800 ha on 18 orchards to the south of Cobram region. The area included 550 ha of peaches and nectarines, which had been treated with MD the previous season. The balance of 250 ha under area-wide MD was comprised of pears, apples, plums and apricots that had not been treated with MD previously. The whole area was considered to be extensive enough to ensure that, within it, any edge effects and hot spots associated with OFM migration would be greatly reduced. Next growing season in 1998, the area-wide MD experiment was expanded to include more than 1,100 ha on 40 orchards. All fruit trees were treated with MD in the area-wide experiment.

Orchards with various pome and stone fruit blocks where OFM was managed with insecticides rather than MD were designated for control. Four control orchards were adjacent to the north, west, east and south borders of the area-wide MD experiment.

When the first area-wide MD project finished in May of 1999, a number of small growers stopped area-wide MD applications and returned to the conventional insecticide spay program on pome fruit with MD treatment of only stone fruit. This resulted in an increase of OFM damage to the neighbouring properties where area-wide MD treatment continued. Affected growers established a community action committee with the support of the Victorian Department of Primary Industries. Finally, in 2001 all Cobram growers decided to continue the area-wide MD program in a self-sufficient community approach. All orchardists in the district joined forces and re-established an area-wide MD across the whole Cobram region in the 2002 season.

The success of the area-wide MD strategy applied by Cobram growers for OFM control became well known to growers in other regions. Growers in East Shepparton and Ardmona areas were experiencing increased pressure from OFM while attempting to utilise MD for CM control in their pome fruit. MD had been successfully used to control either CM or OFM in solid blocks of pome or stone fruit respectively. Although they did not have the advantage of the large closely situated orchard blocks that existed in Cobram, there was a dense enough stand of orchards in areas identified as OFM hot spots to allow area-wide MD approach to work. Two large areas of orchards (about 200 ha each) were established in East Shepparton and a third in Ardmona regions of northern Victoria for the selected area-wide MD treatments (2001-04). In the spring of 2001 these large identified OFM and CM hot spots were entirely treated with MD. Orchards on the edge of the hot spots that did not want to use MD were used as controls. Control orchards were treated with insecticides applied as normal during the season.

Some fruit blocks with high levels of OFM population in the MD treated area were also sprayed with insecticides (usually in the spring) to reduce OFM numbers and allow MD to take control of the remaining OFM population up to the end of the season. Within these hot spots and control areas, the numbers of OFM and CM caught in traps were monitored weekly over three consecutive seasons. The overall effectiveness of an area-wide MD approach was assessed over three seasons (2001-04) by comparing the level of moth infestation from one season to the next in MD treated and control areas.

Dispensers for mating disruption

Dispensers of "Isomate OFM Plus" (Shin-Etsu Chemical Co. Ltd., Japan for Biocontrol Ltd., Australia) were applied high in the tree canopy at a rate of 4 dispensers per tree or 1000 dispensers per ha at the start of the first area-wide MD experiment (1997-98). "Isomate OFM Plus" is a controlled release formulation of polyethylene tubing dispenser with OFM sex pheromone that contains Z-8-dodecenyl acetate (130.3 mg/dispenser), E-8-dodecenyl acetate

(8.4 mg/dispenser) and Z-8-dodecenol (1.3 mg/dispenser). After 1998, "Isomate OFM Rosso" (Shin-Etsu Chemical Co. Ltd., Japan for Biocontrol Ltd., Australia) dispensers were applied at the registered rate of 2 dispensers per tree or 500 dispensers per ha. "Isomate OFM Rosso" is a controlled release formulation of polyethylene tubing dispenser with OFM sex pheromone that contains Z-8-dodecenyl acetate (223 mg/dispenser), E-8-dodecenyl acetate (14.5 mg/dispenser) and Z-8-dodecenol (2.5 mg/dispenser). The "Isomate OFM Rosso" dispenser was used rather than the standard "Isomate OFM Plus" dispenser because of the opportunity to apply a MD formulation at a lower application rate and increased field longevity (Sexton and Il'ichev 2001).

In 2001-04 "Isomate OFM Rosso" was applied for OFM control and "Isomate CTT" (Shin-Etsu Chemical Co. Ltd., Japan for Biocontrol Ltd., Australia) was applied for CM control during selected area-wide MD treatments (2001-04). Isomate CTT is a controlled release formulation of double tubing dispenser with CM sex pheromone that contains E-8, E-10-dodecadien-1-ol (215 mg/dispenser), dodecanol (120 mg/dispenser) and tetradecanol (27.5 mg/dispenser). The registered and recommended application rate of 500 dispensers per ha, or an average of 2 dispensers per tree was applied by the middle of September of each season. The same treatments were applied to the same areas during three consecutive seasons from 2001 to 2004.

Monitoring with food and sex pheromone traps

Food traps (Efecto-fly trap, Avond Pty.Ltd., Western Australia) filled with 1 L of 10% brown sugar solution and 12 drops of terpinyl acetate solution (48.5 mL of terpinyl acetate with 1.5 mL of non-ionic wetting agent and 50 mL of warm water) were used to monitor the population of both male and female of OFM in fruit blocks under MD and in control blocks without MD. The food traps were monitored weekly by collecting moths and changing the sugar and terpinyl acetate solutions.

At least one food trap was placed in all blocks of each fruit variety inside each orchard that was part of the area-wide MD. Additional food traps were placed into blocks larger than 3 ha and overall the average trap density was one trap per 4 ha. More than 230 food traps were placed in the area for monitoring in 1997 and the following season the number of traps was increased to 280 to accommodate an increase in size of the experimental area. Traps were in place prior to the start of OFM flights in the middle of August and weekly monitoring continued for at least two weeks after the last OFM moths were captured in April.

To monitor CM populations in the fruit block treated with MD, Pherocon Delta VI traps (Trece Ltd., Salinas, CA, USA) with 10 mg sex pheromone dispensers were used. These traps were placed on poles as high as possible within a tree canopy. Each Delta trap for CM monitoring was placed in a tree approximately 72 m away from the nearest sex pheromone trap for OFM to minimise interference. All CM traps were also monitored once a week and numbers of CM were recorded. Monitoring information about the OFM and CM numbers caught in food and sex pheromone traps was collected and prepared for analysis. The only records from the same traps placed in the same orchard block during a number of consecutive seasons were used for analysis to make the information comparable across growing seasons.

Management and analysis of data

A geographic information system (GIS) was used for the management, visualisation and analysis of the monitoring and damage assessment data from all area-wide MD experiments. The location of orchard blocks and traps were entered into the GIS using sketch maps of each property in conjunction with satellite imagery and digital cadastral information for the area-wide MD experiments. Once all of the monitoring data had been entered, a desktop GIS package *ArcView 3.1*® (ESRI Inc. USA) was used to locate and interpret the data with respect to cadastre of the Cobram and Greater Shepparton areas and the road network. The GIS data

base was also used to provide all participating growers with weekly reports of OFM numbers monitored on their property and regularly inform them about the situation with outbreaks and hot spots over the whole experimental area.

Results and discussion

Initial area-wide MD experiment in Cobram (1997-99)

The initial area-wide MD experiment, which was established during 1997-99 by treating every fruit tree in the entire experimental area with OFM sex pheromone dispensers for MD, substantially improved protection against OFM damage.

Preliminary monitoring during the 1996-97 season revealed four properties with high OFM populations on the edges. Also during this season, high catches indicated that there were hot spots in two properties within the experimental area. The average trap catch in the whole area was between 5-10 moths/trap/week (m/t/w), but in hot spots it was much higher. Monitoring data of the first OFM flight in 1997-98 confirmed two distinct hot spots in two properties. In the hot spot on property 1, the initial OFM population level on peaches was high, with a peak of the first generation flight of about 85 m/t/w in 1996-97, when adjacent pears were treated with insecticides. In 1997-98, when adjacent pears were treated with MD, the peak of the first generation OFM flight was about 45 m/t/w. Than OFM numbers decreased in the second generation and did not show any increase up to the end of the season, although the numbers continued at a level of 10-20 m/t/w (Figure 1).

The monitoring results of the 1998-99 season indicated that the area-wide MD experiment during the second year successfully reduced OFM in the hot spot in property 1. During the second season of the area-wide MD application the population in the first flight in this hot spot in the 1998-99 season was about 20 m/t/w. Then the OFM population declined to a very low level of 0-3 m/t/w in the third flight and no moth catches were recorded after the third flight (Figure 1). As a result most of the growers involved in area-wide MD experiment did not spray insecticides against OFM in the1998-99 season.

The control blocks were expected to indicate if there had been a general decline in OFM in the district during the experimental period. The area-wide MD approach was so popular that we had difficulty obtaining suitable control blocks without MD. Only one of the control blocks had sufficient moth number to be suitable for comparison of OFM population trends between seasons. This block was managed with insecticides rather than MD and the OFM population did not decline over the life of the experiment (Figure 2). This result suggested that the reduction in OFM in the hot spots was due to the effect of MD rather than seasonal variations in pest pressure.

Final results indicated that the area-wide MD approach worked effectively and during two consecutive seasons of application was able to control high levels of OFM in hot spots. The area-wide MD experiment shows that the OFM populations in hot spots can be gradually reduced and that migration of mated females in the hot spots and any edge damage effects can be reduced. The area-wide MD application in the first season helped growers to halve the number of insecticide sprays against OFM and in the second season, most of the growers did not spray against OFM at all (Il'ichev et al. 2002).

Re-establishment of the area-wide MD treatments by Cobram growers (1999-2002)

The most obvious reason for the increase in OFM damage in Cobram after some growers stopped MD applications was incomplete MD coverage of all OFM host crops leaving opportunities for mating and dispersal. During the 2000-01 season OFM damage continued to increase in pome fruit blocks treated with conventional insecticide spray programs, probably

because of pesticide resistance, and contributed to damage in neighbouring stone fruit blocks and orchards treated with MD (Sexton and Fox 2002).

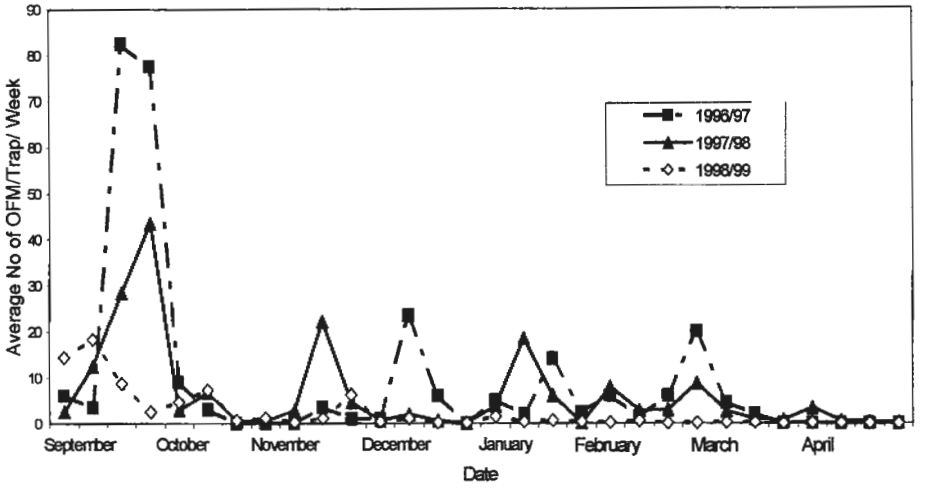

Figure 1. Average number of OFM in peach block under MD in the hot spot area of property 1. Monitoring by food traps was conducted during the 1996-97, 1997-98 and 1998-99 seasons.

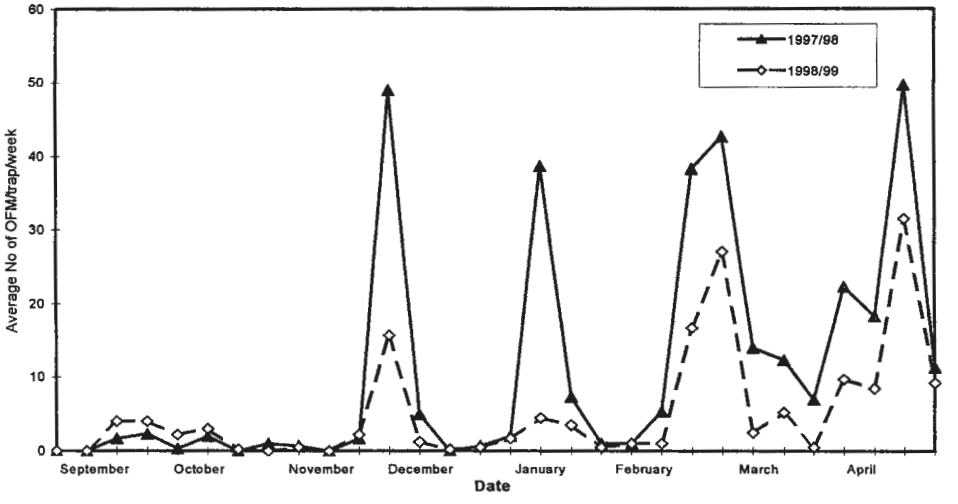

Figure 2. Average number of OFM in control peach block managed with insecticides without MD in the north border of the area-wide MD experiment. Monitoring by food traps was conducted during the 1997-98 and 1998-99 seasons.

Affected growers established an OFM Community Action Committee with the support of government extension specialists. In 2001 all Cobram growers decided to continue the area-wide MD program in a self-sufficient community approach that resulted in most small growers returning to MD application on all stone and pome fruit blocks. Only a limited number of insecticide sprays were applied in spring to reduce the OFM population in the areas with higher infestation level. An area-wide MD had been re-established by all orchardists across the whole Cobram region during the 2001-02 season (Il'ichev et al. 2003).

An extensive media campaign supported the involvement into the area-wide MD program of households and backyard gardens. Keen home gardeners in Cobram town helped the local commercial orchardists to control the dispersal of OFM by applying MD to their home-garden fruit trees. Such broader community co-operation extended the effectiveness of MD coverage to the wider Cobram district and dramatically reduced dependence on insecticides so that the whole community received environmental benefits from area-wide MD application (DPI media release, 2003).

The most significant achievement was that for the first time in the world practically all orchardists in a district were voluntarily involved in an area-wide MD treatment to control OFM. This grower's achievement attracted attention from around the world (Williams and Il'ichev, 2003).

Selective area-wide MD treatments of OFM and CM hot spots (2001-04)

Selective area-wide MD treatments were applied in OFM and CM hot spots during three consecutive seasons from 2001 to 2004, but monitoring in these areas started in the 2000-2001 season, before the area-wide MD experiment. It was important to include previous monitoring information, because the OFM catches indicated the initial level of pest infestation before area-wide MD treatment was applied.

Catches of OFM in the same food trap placed in the same block of plums during 4 consecutive seasons are presented in Figure 3. Monitoring information from 2000-01, before area-wide MD treatment, indicated a very high level of OFM population (more than 80 m/t/w) in autumn (February-March) 2001. This high OFM population level influenced the catches at the beginning of the next growing season in the spring (August-September) 2001, when catches of the first OFM generation peaked at 110 m/t/w. Area-wide MD treatment was applied in early September 2001 and did not influence the first OFM flight, but after November 2001 the number of OFM caught in food traps under MD dramatically decreased to about 30 m/t/w. OFM catches during the following seasons were in general low and only slightly increased in January 2004 (Figure 3).

In one property within the hot spot in East Shepparton before area-wide MD experiment, OFM population started low at the beginning of the season but then increased dramatically and peaked at 150 m/t/w near the end of the growing season in Packham pears (Figure 4). Such tendency for increase of the OFM population level by the end of the season was noticed in the same Packham pears during the first year of area-wide MD application in 2001-02. Then a gradual reduction of OFM numbers was observed during the area-wide MD application. In the third year of area-wide MD application, control of OFM population was achieved in all pome fruit blocks, with average catches of OFM much lower than before the experiment (Figure 4).

The opposite situation was observed in the pome fruit orchards without MD treatment, used as control in the area-wide MD experiment. Dramatic increase in the level of OFM population was recorded during three consecutive seasons in these pear blocks treated with conventional insecticide spray program. In general the OFM population level increased

dramatically by the end of each growing season, reaching high numbers (up to 100 m/t/w) in February and March.

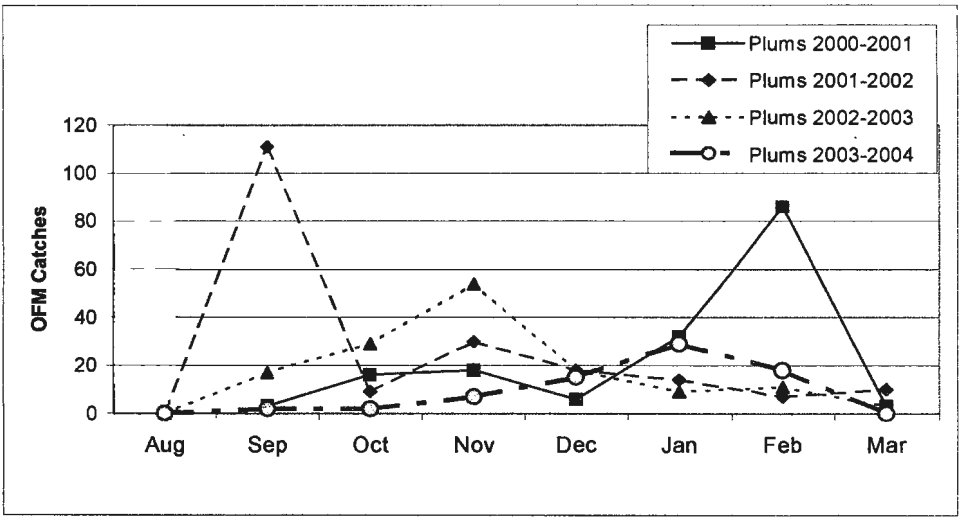

Figure 3. Average number of OFM caught from 3 food traps in a stone fruit (plums) block under MD during 2000–2004 consecutive seasons in East Shepparton region.

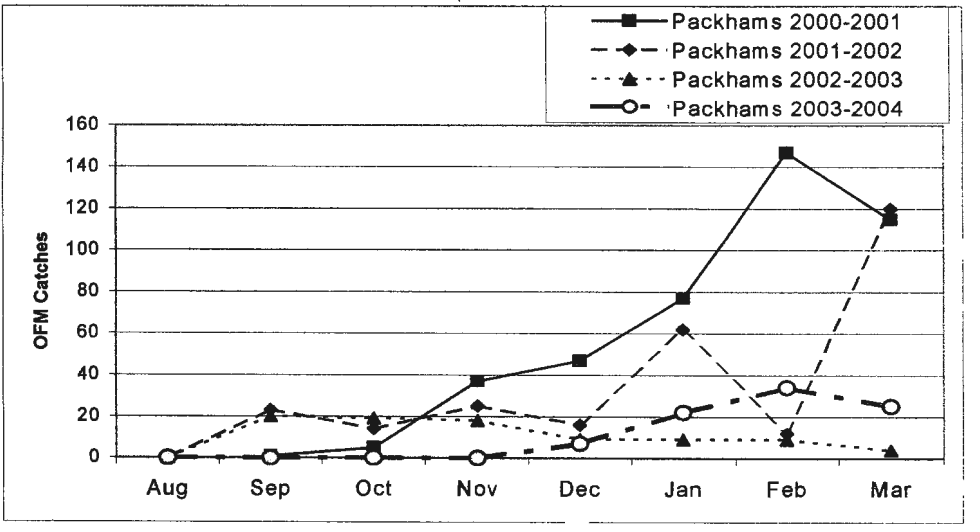

Figure 4. Average number of OFM caught from 3 food traps in a pome fruit (Packhams pears) block under MD during 2000–2004 consecutive seasons in East Shepparton region.

Monitoring data of the codling moth (CM) population in orchards under area-wide MD demonstrated stady decrease of CM catches after the application of an area-wide MD treatment across all orchards in the East Shepparton hot spot (both stone and pome fruit). However, in the control pome fruit blocks without MD treatment a dramatic increase in the level of OFM and CM populations was recorded during three consecutive seasons in the area-wide MD experiment (Il'ichev et al. 2003).

Results collected throughout the Greater Shepparton experimental area demonstrated that application of selective area-wide MD treatments to OFM and CM hot spots during three consecutive seasons provided sustainable control of OFM and CM hot spots. Pheromone-based IPM programs with selective area-wide MD treatments of pest outbreaks promise to be the key elements in the development of cost effective strategies for pest control while protecting the environment by reducing pesticide pressure in orchards.

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Sex pheromone of raspberry cane midge

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Abstract: The sex pheromone of the raspberry cane midge has been identified and synthesised by EMR and NRI and has proved to be highly attractive and useful for pest monitoring. Sex pheromone traps will be available for testing by growers (Beta test) or researchers (Ring test) in 2006 (www.eastmallingresearch.com). For the tests, a nominal threshold of 30 midges per trap is proposed for timing first chlorpyrifos sprays in spring.

Key words: midge blight, pest monitoring, pheromone trap, *Resseliella theobaldi*

Introduction

The raspberry cane midge, *Resseliella theobaldi* (Barnes), is an important pest of raspberry in the UK and in many other areas of the world. The adult midge lays eggs in splits in young canes and larvae feed on the pith beneath the rind causing penetrating lesions which allow entry of diseases such as the cane blight fungus *Leptosphaeria coniothyrium.* There are three or more generations per annum.

The pest is controlled currently by routine sprays of the organophosphorus (OP) insecticide chlorpyrifos applied to control the first generation in spring. This also prevents significant damage by the subsequent generations, although population increase almost certainly occurs. Traditionally, a spray was applied in late April or early May when the spawn was 20-30 cm high, and again about two weeks later. However, more recently a temperature-based forecasting model was developed by Gordon et al. (1989) for predicting spring oviposition by the raspberry cane midge to aid better timing of sprays. Spray warnings are made available to growers by ADAS. First oviposition occurs when a temperature sum of 339°C days above 4°C is accumulated. Values are interpolated from the nearest Meteorological station making a correction for the altitude and aspect of the particular location. The forecast is believed to be accurate to ± 5 days. The problem with this system is that it results in routine use of OP insecticides in most commercial plantations.

The existence of a female sex pheromone in raspberry cane midge had not previously been proven, but it was known that it was likely that one existed, by analogy with related species. The pheromone will be useful for monitoring the pest making the forecasting model redundant and obviating the need for routine sprays. It could possibly be used for control by mating disruption, lure and kill or by mass trapping approaches.

Identification of the raspberry cane midge sex pheromone

Components of the female sex pheromone of the raspberry cane midge have been identified and synthesised by EMR and NRI. Pheromone components were collected by trapping of volatiles and could be detected by comparison of GC analyses of collections from females and males as well as by linked GC-EAG analyses. One major component and three minor

components were identified by interpretation of their mass spectra and comparison with synthetic standards. The major component and two of the minor components have a single chiral centre but only one enantiomer of each is produced by the insect. The major component belongs to a new class of midge pheromone structures that is currently the subject of a patent application.

Attractiveness in the field

In initial field tests in May 2005, delta traps suspended in raspberry plantations at 50 cm height above ground and bated with rubber septa or polythene vial dispensers impregnated with 100 μg of the synthetic, racemic major component of the pheromone, alone or in admixture with the minor components each at 30% of the amount of the major component (the natural ratio), all proved highly attractive to males. There were no obvious differences between the dispensers and no clear benefit from addition of the minor components. In a subsequent experiment, the attractiveness of rubber septa lures loaded with 0, 0.1, 1, 10, 100 and 1000 μg of the racemic major component alone were compared over a 4 week period. The 0.1 μg loading proved highly attractive catching an average of 150 males per trap. The maximum catch occurred with the 100 μg loading there being a significant reduction in catch at the highest loading.

Pest monitoring in 2005

Single white delta traps bated with polythene vial dispensers containing 100 μg of the racemic major component of the pheromone plus 30% of each of the minor components, were deployed in 10 raspberry plantations in Kent and monitored weekly from 10 May to end of September 2005. Plantations comprised a range of varieties grown under protection or in the open field (Table 1). Two plantations at East Malling Research contained a very wide range of varieties (variety collections) and had not received any pesticide sprays. The other plantations were sprayed with pesticides including chlorpyrifos for cane midge control.

Regrettably, the pheromone was not available for deployment until 10 May, 4 days after the forecast date of first emergence by ADAS on 6 May 2005 at East Malling Research.

There was no clear pattern of midge emergence at the 10 sites making it difficult to discern distinct generations of midge emergence. Small numbers of midges were captured in the first week the traps were deployed at all sites. There was evidence of a first generation in May at approximately the time of the ADAS forecast but this was difficult to distinguish and numbers were small compared to numbers that emerged later in May or in June or July (Figure 1, Table 1). There were large differences in the numbers of midges caught, very large numbers (>>1000 over the season) being caught in 5 of the plantations with small numbers (< 1000) in the 5 others. First catches from 10-17 May varied from 1 to 112 midges/trap and were not necessarily a good indication of the magnitude of subsequent total catches.

Advantages of the raspberry cane midge sex pheromone trap

There are many important advantages of the raspberry cane midge sex pheromone trap over the current system of temperature based forecasting of the start of spring emergence. The most important are that records are site/plantation specific and indicate the timing and intensity of midge attack through the season.

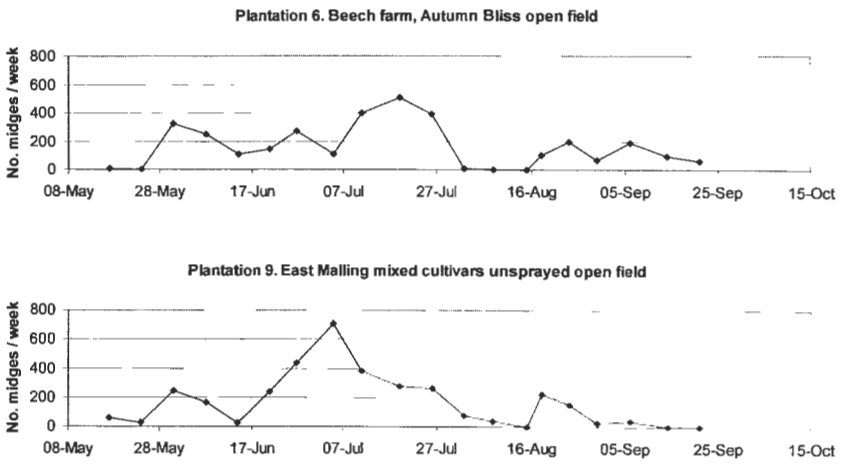

Figure 1. Catches of raspberry cane midge males in a sex pheromone trap in an open field Autumn Bliss plantation at Beech Farm, W. Peckham (above) and in unsprayed raspberry plantation of mixed cultivars at East Malling Research (below) in 2005.

Availability of sex pheromone traps for testing in 2006

The results of work in 2005 clearly show that the raspberry cane midge sex pheromone is highly useful for monitoring midge populations. EMR and NRI have developed a protocol for a collaborative ring test of the sex pheromone trap monitoring system in 2006. Lures and traps will be supplied free of charge to researchers who wish to take part (contact jerry.cross@emr.ac.uk). They will also be available to growers for a Beta test in 2006 at a cost of £50 (ex VAT and additional postal charges outside UK) per trapping station (details and registration www.eastmallingresearch.com or NRI website www.gre.ac.uk/~hd18/chemecol (look under 'Projects' for Raspberry cane midge pheromone)). A trapping station will include:

- A standard white delta trap and hanger
- Six rubber septa lures to be changed monthly for 1 April to 30 September for the trap
- 24 sticky bases to be changed weekly
- Instructions on how to deploy and monitor the traps including a colour photograph to aid midge identification
- Instruction on data collation and relay of results and details of plantations and insecticide applications to EMR

The traps should be deployed from early April to the end of September and the catch recorded weekly. EMR and NRI will collect the data from participants, either from participants directly during the season by email, or at the end of the season.

Table 1. Seasons catches in single raspberry cane midge sex pheromone traps in 10 raspberry plantations in Kent in 2005.

Plantation	Variety	Protected/ open field	Pesticide for cane midge	Total no. of midges caught in single trap						
				10-17 May	May	June	July	Aug	Sep	Total
1. Belks fm, Otham	G. Ample	Open	Yes	16	17	20	117	112	24	290
2. Belks fm, Otham	G. Lion	Protected	Yes	7	44	210	123	166	29	572
3. Belks fm, Otham	G. Ample	Open	Yes	112	123	73	341	76	0	613
4. Belks fm, Otham	G. Lion	Protected	Yes	11	12	71	170	185	80	518
5. Beech fm, W. Peckham	J Squire	Open	Yes	3	283	749	1129	1724	259	4144
6. Beech fm, W. Peckham	A Bliss	Open	Yes	7	333	769	1406	370	338	3216
7. Beech fm, W. Peckham	G Ample	Protected	Yes	97	208	826	1284	3944	1018	7280
8. Beech fm, W. Peckham	G Ample/Tulameen	Open	Yes	1	13	265	149	256	72	755
9. East Malling Research	Mixed	Open	No	61	335	863	1635	523	37	3393
10. East Malling Research	Mixed	Open	No	12	36	253	465	803	70	1627

Interpretation of trap catches for 2006

No scientific work has yet been done on the relationship between pheromone trap catches, time of season, crop susceptibility and the intensity of larval attack. No firm guidance thus can be given on the use of the trap catches to time sprays for large scale commercial purposes. For the tests, a nominal trap threshold of 30 midges per trap in May to trigger chlorpyrifos application is proposed but the authors accept no liability for the efficacy of this approach.

Future R&D

The results to date have thus provided a basis for development of a much-needed monitoring tool for cane midge. A UK Hort LINK project will fund this and future field investigations into the cane midge sex pheromone. The Beta test results will provide data for correlation catches in the pheromone traps with field populations of the midge. Work will start in 2006 to investigate controlling the pest by mass trapping, attract-and-kill and/or mating disruption, avoiding or at least minimising use of conventional insecticides.

Acknowledgements

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Preliminary study of tebufenpyrad resistance in the two spotted spider mite *Tetranychus urticae* Koch in Swiss strawberry and raspberry production

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Abstract: Four strains of *Tetranychus urticae* collected in 2004 in Valais (Switzerland) were treated with varying doses of the acaricide tebufenpyrad to check for resistance. Two out of the four tested strains, one from strawberry and the other from raspberry, proved to be highly resistant to tebufenpyrad. The respective ratios of the LD_{50} of each strain to that of the sensitive strains were 1720 and 694. The two other strains expressed a sensitivity identical or close to that of the sensitive strain. This is the first case of resistance in *T. urticae* to tebufenpyrad observed in Switzerland. The implications of this first resistance record are briefly discussed.

Keywords: tebufenpyrad, *Tetranychus urticae,* raspberry, resistance, strawberry

Introduction

During the nineties, three new acaricides belonging to the new chemical group of METIs (Mitochondrial Electrons Transport Inhibitors) made their appearance on the Swiss pesticide market: tebufenpyrad, fenpyroximate and fenazaquin. Over the past few decades, METIs, especially tebufenpyrad which has effect against all stages of *Tetranychus urticae* Koch, have been widely used by soft fruit growers. Repeated treatments with compounds from the same chemical group can lead to the target quickly building up resistance, and cases of *T. urticae* resistance to tebufenpyrad have been established in numerous countries worldwide (Australia, Korea, Japan, England, Belgium, etc.) (Herron & Rophail 1998, Devine et al. 2001, Stumpf & Nauen 2001). Recent control breakdowns in the Valais region (Switzerland) led the authors to suspect that *T. urticae* had developed a certain level of resistance to METIs. In order to clarify whether these failures were due to resistance or to inadequate application technique, strains of *T. urticae* originating from farms which had encountered major control failures over the past years were tested for resistance to tebufenpyrad under laboratory conditions.

Materials and methods

Strains

Table 1 shows the main characteristics of the plots where the *T. urticae* strains were collected. All strains were reared on bean leaf discs (6 cm) disposed on wet cotton wool in open Petri dishes (Guttierez 1994, Helle & Overmeer 1985). Petri dishes were placed in aerated plastic boxes at 25°C, 70% RH, and a photoperiod of 16:8 (L:D). In order to avoid age variability all the tests were conducted on unmated females of the same age (± 24 hours). The tests took place within 2 months after field collection.

Table 1. Main characteristics of the plots where *T. urticae* strains were collected in 2004.

Strains	Crop	Type of Production	Variety	Surface area (m^2)
1. S strain	Strawberry	Private garden (Organic)	Mara des Bois Wädenswil	10
2. St Léonard	Strawberry	Plastic tunnel (Organic)	Madeleine	20000
3. Ardon 1	Strawberry	Plastic tunnel (IPM)	Arosa	5000
4. Ardon 2	Strawberry	Plastic tunnel (IPM)	Elsinor	5000
5. Ardon 3	Raspberry	(IPM)	Tulameen	20000

Bioassays

Tebufenpyrad 20% wettable powder (WP) was the only acaricide tested. The product was tested using the leaf disc residue-dipping method adapted from Kabir et al. (1993). Leaf discs were dipped for 5 seconds in the tebufenpyrad solution, placed in Petri dishes containing wet cotton wool and allowed to dry at room temperature. Ten females per disc were then released (3 discs per dose). Each test was replicated two times and included a water treated control which did not exceed 15% mortality. The sprayed discs with mites were then kept at 25°C and mortality was recorded every 24 hours for 3 days. Mites were considered dead if they were unable to get back on their legs when put on their back with a fine brush. Run-off mites were considered as dead. The strains were first tested at the rates of 0.8, 1, 2, 4, 8 and 80 ppm active ingredient (the latter being the maximum registered dose in Switzerland). If necessary, they were then tested against 3 more concentrations to allow the calculation of log-dose probit regressions with the help of the POLO computer programme (Roberston & Preisler 1992).

Results and discussion

The main results are given in Table 2.

Table 2. Dose-response data for tebufenpyrad against 5 different strains of *T. urticae.*

Strain	Linear Regresssion	LD_{50} (ppm)	Confidence Interval (90%)	LD_{90} (ppm)	Confidence Interval (90%)
1.	y=2.29x - 0.73	2.08	(1.37-2.80)	7.56	(5.28-14.37)
2.	y=2.06x - 0.32	1.43	(0.95-1.97)	6.01	(3.94-13.44)
3.	y=2.73x - 0.02	1.02	(0.76-1.25)	3.00	(2.36-4.34)
4.	y=0.97x - 3.46	3577.9	(1435.70-6912.60)	74031	(29364-522650)
5.	y=0.96x - 3.05	1444.3	(742.74-2460.62)	30713	(16903-73231)

Looking at the LD_{50} levels, two different groups can be observed. The first three strains show similar responses to tebufenpyrad with values ranging from 1 to 2 ppm, while the last 2 strains show very high values. The first group comprises strains still highly susceptible to the registered dose of 80 ppm. With LD_{90}s 4,000 to 10,000 times higher than that of the S strain, the females of the second group can be considered as highly resistant. Figure 1 illustrates the two different groups obtained.

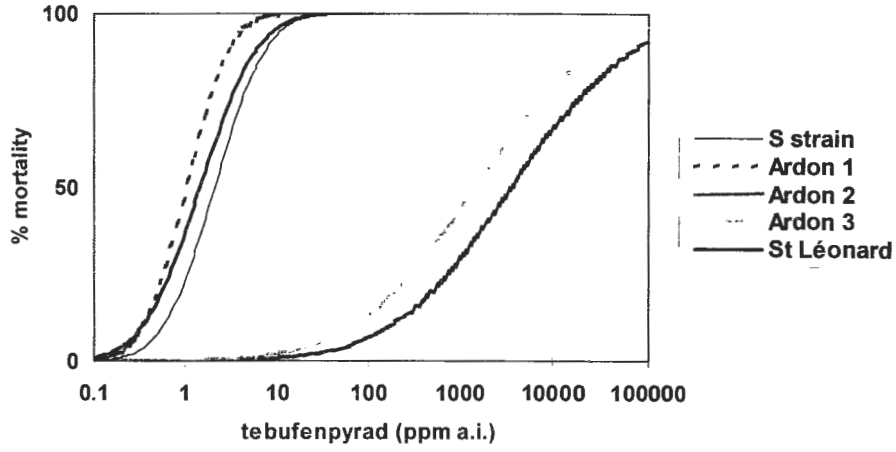

Figure 1. Dose-Response for the 5 strains of *T. urticae* against tebufenpyrad.

Even if different factors (test method, mortality estimation, low numbers of females tested, etc.) could have had an effect on the results of this preliminary study, the considerable difference between the two groups is a clear indication that tebufenpyrad resistance has evolved in the mites from Ardon 2 and 3. These two plots are contiguous and it was therefore not surprising that the mites originating from these fields had similar test results. The raspberry field was planted in 2002 immediately after a strawberry crop which had been treated once with tebufenpyrad. The next two years the raspberries received two additional treatments. It is therefore possible that resistance appeared after only 3 sequential treatments. The same phenomenon was reported by Herron & Rophail (1998) who observed resistance after 4 applications in an apple orchard. The resistant strain found in the nearby strawberries planted in the spring of 2004 probably came from the raspberry plot. Resistance is more likely to show up in perennial crops like raspberries than in annual crops.

These observations are the first reported case of tebufenpyrad resistance in *T. urticae* in Switzerland. It should lead growers to adopt better resistance management strategies to assure the longest possible "life" to METI products. In situations where tebufenpyrad still gives satisfactiory results it is absolutely necessary that treatments are alternated with other products from other groups. The recent registration of a new acaricide (spiridoclofen) from a new group (tetronic acids) on strawberries offers an interesting alternative. In raspberries, biological control with native or introduced predatory mites has given some good results and should help to diminish the need for chemical treatments.

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Envidor 240 SC – a new acaricide and its efficacy in controlling strawberry mite (*Phytonemus pallidus* ssp. *fragariae* Zimm.) and two-spotted spider mite (*Tetranychus urtiace* Koch) on strawberry in Poland

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Abstract: Experiments with the new acaricide Envidor 240 SC (spirodiclofen) were carried out in the last few years in strawberry plantations in Skierniewice, Poland. The aim of these trials was to evaluate the ability of this acaricide to control two major strawberry pests: strawberry mite (*Phytonemus pallidus* ssp. *fragariae* Zimm.) and two-spotted spider mite (*Tetranychus urtiace* Koch). The rates of Envidor 240 SC used against strawberry mite were 0.4 and 0.6 l per ha. Sprays were applied twice after strawberry harvest with a one week interval. Effects of treatments were assessed 4-5 weeks after application. The results obtained were similar to those with standard chemical amitraz (Mitac 200 EC) and somewhat poorer than those obtained with second standard endosulfan (Thiodan 350 EC). Due to imminent withdrawal of popular chemicals such as amitraz and endosulfan there is a constant need to search for new acaricides to control this serious strawberry pest.

Envidor 240 SC was used against two-spotted spider mite at lower rates of 0.3 and 0.4 l per ha. A single spray was applied in spring, before the blossom, or in summer, after harvest of strawberry. The results were good and satisfactory. The number of mites observed on strawberry plants after Envidor 240 SC treatment was very similar to those of the standard acaricides propargite (Omite 30 WP) and hexythiazox (Nissorun 050 EC). The results obtained showed that Envidor 240 SC after registration could be useful for control of both strawberry pests. It would also be suitable to protect strawberry plantations in Integrated Fruit Production (IFP) programmes.

Key words: acaricides, *Phytonemus pallidus*, strawberry, *Tetranychus urticae*

Introduction

Strawberry mite and two-spotted spider mite are very important pests of strawberry (Alford 1972, Łabanowska 2000, Łabanowska 2003). All strawberry cultivars tested in Poland are infested with these pests (Łabanowska 2004, Łabanowska and Bielenin 2002) and they need to be continuously controlled. Recently, two main active ingredients such as endosulfan and amitraz, usually used to control strawberry mite in Poland have already been or are going to be withdrawn soon (Łabanowska 2005) This is the reason why new acaricides to control these pests need to be found. Controlling two-spotted spider mite is still less of a problem than controlling strawberry mite. Some acaricides such as fenbutatin oxide (Torque 50 WP) and amitraz (Mitac 200 EC) have been withdrawn but some new formulation have already been tested (Łabanowska and Maciesiak 1996, Łabanowska 1995). A number of fungicides used against fungal disease caused by *Botritis cinerea* also reduce the mite population (Meszka et al. 2000, Łabanowska and Meszka 2003).

The aim of this work was to evaluate the efficacy of the new acaricide spirodiclofen (Envidor 240 SC, manufactured by Bayer) for control of strawberry mite and two-spotted spider mite on strawberry. Spirodiclofen belongs to a new class of chemicals (Nauen et al.

2000) and has given good control of two-spotted spider mite on black currant in Poland (Łabanowska 2002).

Material and methods

***Control of the strawberry mite* (Phytonemus pallidus *ssp.* fragariae *Zimm.*)**
The experiments were conducted in 2001- 2003 at the Research Institute of Pomology and Floriculture in Skierniewice. They were located on 2-3 years old strawberry plantations, cultivar Senga Sengana. Each experiment was designed in the strip system, where a plot about 66.5 square meters (7 rows, 10.5 m long) of strawberry plantation constituted one treatment, subdivided into four plots (7 rows, 2.6 m long). A knapsack motor sprayer 'Stihl' was used to apply the acaricide at the rate 5.0 l per combination, equal to 750 l of spraying liquid per ha. Two treatments, at a one week interval, were applied in the summer, after harvest of strawberry. The strawberry mite population density was estimated just before the first treatment and 4-5 times, at one week intervals, after the second spraying. The motile forms of mites and eggs were counted separately on leaves, under a stereoscopic microscope. Samples of 10 leaves were taken randomly from each replication. The numerical data was analysed statistically by analysis of variance. Duncan's multiple range tests was used for separation of means at the 5 % significance level.

***Control of the two-spotted spider mite* (Tetranychus urticae *Koch*)**
The experiments were conducted in 2001- 2002 at the Research Institute of Pomology and Floriculture in Skierniewice. They were located in 2-3 years old strawberry plantations, cultivar Senga Sengana. Each experiment was designed in the strip system, where a plot about 66.5 (2001) or 133 (2002) square meters in area (7 or 14 rows, 10.5 m long) of strawberry plantation constituted one treatment, subdivided into four plots (7 or 14 rows, 2.6 m long). A knapsack motor sprayer 'Stihl' was used to apply the acaricide at the rate 5.0 l or 10 l per combination, equal to 750 l of spraying liquid per ha. One treatment was applied either in spring or in summer, before bloom or after harvest of strawberry. The two-spotted spider mite population density was estimated just before spraying and after later on 4 times every 1 or 2 weeks. The active stages of mites and eggs were counted separately using Henderson's and McBurnie's (1943) technique. Samples of 30 leaves were taken randomly from each plot in four replications. The numerical data was analysed statistically. Duncan's multiple range test was used for separation of means. The Cumulative Indices of Infestation (CII) was calculated according to Wratten's et al. (1979) formula, and then recalculated into percentage assuming CII for untreated control = 100.

Results and discussion

***Control of the strawberry mite* (Phytonemus pallidus *ssp.* fragariae *Zimm.*)**
In the first trial in 2001, Envidor 240 SC at the lower rate 0.4 l/ha applied twice, with one week interval, in summer after harvest of strawberry, provided good control of strawberry mite during 5 weeks after second treatment (Table 1a). The number of motile forms of *Phytonemus pallidus* was lower than 1 mite/leaf. Envidor 240 SC applied in the same way, but at the higher rate 0.6 l/ha, showed somewhat poorer results compared with the lower rate. The results obtained after using Envidor 240 SC were worse than after treatment with the standard acaricide Thiodan 350 EC at the rate 2.5 l/ha. But on the other hand the results with

Table 1: Average number of the strawberry mite *Phytonemus pallidus* ssp. *fragariae* per strawberry leaf, Sierakowice, 2001.

a) active stages

Acaricide*	Rate l per ha	Weeks after 2-nd treatment and dates				
		1 Aug.13	2 Aug. 20	3 Aug. 27	4 Aug. 30	5 Sept.10
Envidor 240 SC	0.4	1.2 b**	0.2 b	0.02 a	0.8 b	0.2 b
Envidor 240 SC	0.6	3.1 c	0.3 b	3.9 c	17 c	2.1 c
Mitac 200 EC	4.0	0.3 a	0.6 b	1.0 b	1.0 bc	0.1 b
Thiodan 350 EC	2.5	1.2 b	0.0 a	0.0 a	0.06 a	0.0 a
Untreated control	-	11.3 d	4.9 c	5.7 c	6.3 d	5.9 d
b) eggs						
Envidor 240 SC	0.4	1.6 b	0.2 ab	0.03 a	0.8 b	0.3 b
Envidor 240 SC	0.6	4.3 c	0.4 bc	4.9 c	2.2 c	2.2 c
Mitac 200 EC	4.0	0.3 a	0.9 c	1.2 b	1.3 bc	0.2 b
Thiodan 350 EC	2.5	1.2 b	0.0 a	0.0 a	0.06 a	0.0 a
Untreated control	-	15.0 d	6.5 d	8.6 d	9.4 d	7.9 d

Before treatment there were 2-3 active stages and 3-5 eggs/leaf.
Dates of treatments: July 30 and August 6, 2001
*Envidor 240 SC (spirodiclofen), Mitac 200 EC (amitraz), Thiodan 350 EC (endosulfan).
**Means followed by the same letter are not significantly different (P=0.05), Duncan's multiple range test.

Table 2: Average number of the strawberry mite *Phytonemus pallidus* ssp. *fragariae* Zimm. per strawberry leaf, Sierakowice 2002.

a) active stages

Acaricide	Rate l per ha	Weeks after 2-nd treatment			
		1 July10	2 July 17	3 July 24	4 July31
Envidor 240 SC	0.4	4.0 c	1.9 c	1.9 c	0.9 b
Envidor 240 SC	0.6	3.0 c	0.6 b	1.8 c	0.9 b
Envidor 240 SC + wetting agent	0.4	2.5 c	2.5 c	1.1 c	0.6 b
Mitac 200 EC	3.0	0.5 b	1.4 c	0.5 b	0.7 b
Thiodan 350 EC	2.5	0.1 a	0.1 a	0.1 a	0.1 a
Untreated control	-	15.9 d	14.1 d	9.6 d	7.8 c
b) eggs					
Envidor 240 SC	0.4	5.0 c	1.9 b	2.2 c	2.2 c
Envidor 240 SC	0.6	3.6 c	1.2 b	2.1 c	0.7 b
Envidor 240 SC + wetting agent	0.4	3.4 c	2.3 b	1.0 bc	0.5 ab
Mitac 200 EC	3.0	0.4 b	2.6 b	0.6 b	0.7 b
Thiodan 350 EC	2.5	0.02 a	0.04 a	0.3 a	0.2 a
Untreated control	-	34.1 d	27.9 c	18.4 d	15.2 d

Before treatment there were 10-13 mites and 25-40 eggs/leaf.
Dates of treatments: June 26 and July 3, 2002.
Other explanations as in Table 1.

Table 3. Average number of the strawberry mite *Phytonemus pallidus* ssp. *fragariae* Zimm. per strawberry leaf, Góra Puławska 2003.

a) active stages

Acaricide	Rate l per ha	Weeks after 2-nd treatment 1	2	3	4
Envidor 240 SC	0.4	10.1 c	5.1 c	5.4 c	19.8 d
Envidor 240 SC	0.6	4.4 b	4.0 c	6.1 c	9.1 c
Envidor 240 SC + wetting agent	0.6	3.8 b	3.6 c	6.8 c	3.3 b
Mitac 200 EC	3.0	4.2 b	1.2 b	1.2 b	2.2 b
Thiodan 350 EC	2.5	0.02 a	0.4 a	0.5 a	0.8 a
Untreated control	-	25.1 d	23.2 d	22.1 d	22.1 e

b) eggs

Envidor 240 SC	0.4	16.7 c	8.9 c	13.2 c	29.3 c
Envidor 240 SC	0.6	7.7 b	8.1 c	13.5 c	11.9 bc
Envidor 240 SC + wetting agent	0.6	5.6 b	7.1 c	16.5 c	6.2 b
Mitac 200 EC	3.0	6.5 b	1.7 b	2.4 b	5.6 b
Thiodan 350 EC	2.5	0.03 a	0.5 a	0.5 a	1.3 a
Untreated control	-	60.0 d	52.8 d	42.3 d	54.1 d

Before treatment there were 23-29 mites and 55-69 eggs/leaf.
Dates of treatments: July 15 and 22. 2003.
*Explanations see under Table 1.

Table 4. Average number of two-spotted spider mite, *Tetranychus urticae* per strawberry leaf, Sierakowice Prawe 2001.

a) active stages

Acaricide	Rate l, kg/ha	Weeks after treatment and dates 1 June 1	2 June 8	3 Jun.16	5 Jun.29	CII (Control =100)
Envidor 240 SC	0.3	0.1 a	0.2 a	0.04 a	0.08 a	1.1 a
Envidor 240 SC	0.4	0.4 b	0.5 a	0.3 b	0.1 a	2. a
Nissorun 050 EC	0.9	1.8 c	1.2 b	0.3 b	0.9 b	7. a
Omite 30 WP	2.25	0.05 a	0.2 a	0.4 b	1.8 b	5. a
Untreated control	-	9.0 d	10.2 c	10.9 c	15.9 c	100. b

b) eggs

Envidor 240 SC	0.3	0.4 a	0.3 a	0.1 a	0.4 a	0.7 a
Envidor 240 SC	0.4	1.3 b	0.5 a	0.5 b	0.7 a	1.8 a
Nissorun 050 EC	0.9	5.9 d	5.0 b	13.1 c	4.0 b	18.8 b
Omite 30 WP	2.25	1.9 c	0.5 a	0.7 b	5.9 b	7.0 a
Untreated control	-	31.4 e	47.9 c	66.9 d	15.4 c	100.0 b

Before treatment there were 3-5 mites and 14-19 eggs/leaf.
Date of treatment: May 25, 2001.
Nissorun (hexythiazox), Omite (propargite).
*Other explanations see under Table 1.

Table 5. Average number of twospotted spider mite, *Tetranychus urticae* per strawberry leaf, Miedniewice 2001.

a) active stages

Acaricide	Rate l, kg/ha	Weeks after treatment and dates				CII (Control =100)
		1 June 7	2 June 14	3 Jun.21	5 July 5	
Envidor 240 SC	0.3	0.3 b	0.05 a	0.04 a	0.1 a	5.7 a
Envidor 240 SC	0.4	0.3 b	0.4 c	0.4 b	0.2 a	15.0 b
Nissorun 050 EC	0.9	0.2 b	0.08 ab	0.04 a	0.3 a	7.0 ab
Omite 30 WP	2.25	0.04 a	0.3 bc	0.1 ab	0.09 a	8.0 ab
Untreated control	-	1.7 c	2.5 d	2.5 c	2.1 b	100.0 c

b) eggs

Envidor 240 SC	0.3	0.7 c	0.6 a	0.3 ab	0.2 a	10.1 ab
Envidor 240 SC	0.4	0.4 ab	0.7 a	0.4 b	0.5 b	13.2 bc
Nissorun 050 EC	0.9	0.4 b	1.0 a	0.9 bc	0.4 ab	18.5 c
Omite 30 WP	2.25	0.09 a	0.4 a	0.2 a	0.3 ab	6.2 a
Untreated control	-	2.7 d	3.9 b	5.7 d	3.3 c	100.0 d

Before treatment there were 1.5-2.5 mites and 2.7-3.1 eggs/leaf.
Date of treatment: May 31 2001.
Explanations see under Table 1 and 4.

Table 6. Average number of twospotted spider mite, *Tetranychus urticae* per strawberry leaf, Miedniewice 2002.

a) active stages

Acaricide	Rate l, kg/ha	Weeks after treatment and dates				CII (Control=100)
		1	2	3	5	
Envidor 240 SC	0.3	2.1 a	1.3 b	0.9 b	0.05 a	10.3 b
Envidor 240 SC	0.4	15 a	1.3 b	0.6 b	0.08 a	9.8 b
Envidor 240 SC + wetting agent	0.3	1.5 a	0.5 a	0.5 ab	0.25 b	9.1 ab
Nissorun 050 EC	0.9	1.6 a	0.7 ab	0.2 a	0.3 bc	6.1 a
Omite 30 WP	2.25	2.2 a	0.8 b	0.4 ab	0.4 bc	9.1 ab
Untreated control	-	13.3 b	12.0 c	9.2 c	1.5 d	100.0 c

b) eggs

Envidor 240 SC	0.3	4.1 b	2.3 b	1.0 c	0.02 a	8.6 b
Envidor 240 SC	0.4	1.1 a	2.5 b	1.0 c	0.4 bc	7.1 b
Envidor 240 SC + wetting agent	0.3	0.9 a	0.5 a	0.5 b	0.7 bc	4.9 ab
Nissorun 050 EC	0.9	4.5 b	2.3 b	0.3 a	0.3 b	8.0 b
Omite 30 WP	2.25	1.0 a	0.4 a	0.4 ab	0.6 bc	3.7 a
Untreated control	-	22.4 c	28.2 c	21.3 d	2.9 d	100.0 c

Before treatment there were 9-13 mites and 25-29 eggs/leaf.
Date of treatment 26.06.2002.
Explanations see under Table 1 and 4.

Envidor 240 SC were similar to those obtained with other standard acaricide Mitac 200 EC at the rate 4.0 l/ha. The strawberry mite population on strawberry plants sprayed with Envidor 240 SC at the both rates, during 5 weeks of observations, was much lower than on untreated plants. On the untreated control plants, 4.9-11.3 mites per leaf were noted in August and 5.9-6.3 mites per leaf at the beginning of September. The eggs population was correlated with the number of active stages (Table 1b).

On the plants treated with Envidor 240 SC during observation time the mites were noted on 16-27 % of leaves. On plants sprayed with standard Mitac 200 EC mites were observed on 18.5 % of leaves, whilst only on 7.0 % of leaves on plants treated with other standard, Thiodan 350 EC. In the untreated control plots the mites were observed on 45.5 % of leaves.

In the second trial in 2002, acaricide Envidor 240 SC at both rates, 0.4 l/ha and 0.6 l/ha, and at the lower rate 0.4 l/ha + wetting agent (surfactant), gave similar results (Table 2a). These results were also similar to those obtained on plants sprayed with standard acaricide Mitac 200 EC at 4.0 l/ha. The standard acaricide Thiodan 350 EC at 2.5 l/ha showed the best results, very similar to those from the first trial. On all treated plants, the number of the strawberry mite was much lower than on untreated plants. Number of eggs was correlated with active stages population (Table 2b).

In the other trial with acaricide Envidor 240 SC at the rate 0.4 l/ha and 0.6 l/ha and Envidor 240 SC at the rate 0.4 l/ha + wetting agent (surfactant) during three weeks after 2-nd treatment results were similar (Table 3a). However in the 4-th week after treatment the number of mites was very high on plants treated with lower rate of acaricide Envidor 240 SC, the number of mites increased. In this trial the number of strawberry mite on plants treated with Envidor 240 SC at the beginning of experiment was similar to this on plants treated with standard Mitac 200 EC. Later on, the results with mentioned standard were usually better than after using Envidor 240 SC. The best results were obtained with standard Thiodan 350 EC. On all treated plants the number of strawberry mite was much lower than on untreated check strawberry plants. The number of eggs was correlated with active stage mite population (Table 3b).

Generally, Envidor 240 SC used to control the strawberry mite on strawberry showed results similar or only a little poorer comparing with standard acaricide Mitac 200 EC. The results obtained with Envidor 240 SC confirmed those from the first experiment (Łabanowska 2003). Thiodan 350 EC was the best acaricide, as usually (Łabanowska 1992, 2003). But both acaricides used as standard are due to be withdrawn from the use against the strawberry mite. In this situation Envidor 240 SC after registration could be used to control *Phytonemus pallidus* on strawberry.

***Control of the two-spotted spider mite* (Tetranychus urticae *Koch*)**

In trials 2001 Envidor 240 SC at the both rates, 0.3 and 0.4 l/ha, applied once just before blossom, provided good and satisfactory control of two-spotted spider mite for at least 5 weeks after treatment (Table 4-5). The number of active stages (and eggs) of mites was lower than 1 mite per leaf (the economic threshold level in June is 3 mites per leaf). Results obtained after using Envidor 240 SC were better or similar to those with standard acaricides – Nissorun 050 EC and Omite 30 WP. Number of mites on untreated (check) plants in the first experiment was very high, but it was much lower in the 2-nd trial (Table 5). In the next experiment (2002), Envidor 240 SC used against high population of two-spotted spider mite after harvest gave good control of the pest (Table 6). The mite population after treatment was below the economic threshold level. Results were similar to those obtained with standard acaricides – Nissorun 050 EC and Omite 30 WP. The mite population (active stages and eggs) was much lower than on check plants.

Generally Envidor 240 SC used at the rate 0.3 and 0.4 l/ha and 0.3 l/ha + wetting agent gave good control of the two-spotted spider mite on strawberry. These results confirmed the earlier ones with control the two-spotted spider mite on black currant (Łabanowska 2002).

Conclusions

Acaricide Envidor 240 SC at the rate 0.4 l/ha and 0.6 l/ha applied twice (with one week interval) after harvest of strawberry provided good reduction of the strawberry mite, *Phytonemus pallidus* ssp. *fragariae* Zimm. on strawberry. The results after using of Envidor 240 SC were similar to those obtained with standard Mitac 200 EC and poorer than those obtained with other standard Thiodan 350 EC (but both of them are due to be withdrawn from using in plant protection). The acaricide Envidor 240 SC at the rate of 0.3 l/ha, 0.4 l/ha and 0.3 l/ha + wetting agent applied before blossom or after strawberry harvest provided very good control of the two-spotted spider mite, *Tetranychus urticae* Koch on strawberry. The results with Envidor 240 SC were similar to those with standard Nissorun 050 EC and Omite 30 WP.

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Integrated Pest Management of pests of raspberry (*Rubus idaeus*) – possible developments in Europe by 2015

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Abstract: In this paper we will attempt to predict developments in Integrated Pest Management (IPM) of insect and mite pests of raspberry in Europe over the next decade. The raspberry industry is very dynamic and currently evolving at a considerable rate. New growing practices are being adopted and local industries are restructuring to meet the market pressures from competitors, processors, supermarkets and consumers. Being in such a 'fluid state' makes predictions more difficult, so IPM researchers need to keep up with the changes, or indeed, help to drive the changes in the industry. To do so also requires 'crystal-ball-gazing' based on many years of experience in cane and bush fruit research. Future management of the major pests of raspberry e.g. raspberry beetle (*Byturus tomentosus*), raspberry aphids (*Amphorophora idaei* and *Aphis idaei*), raspberry cane midge (*Resseliella theobaldi*) and two-spotted spider mite (*Tetranychus urticae*) will be considered, as will minor pests, e.g. wingless weevils (*Otiorhynchus* spp.), small raspberry sawfly (*Priophorus morio*) and the cantharid beetle (*Cantharis obscura*). The role of 'new pests' that can colonise the crop, either with the introduction of new cultivars or changes in overall crop management or pest and disease control strategies, will also be discussed. The introduction of protected and/or semi-protected (tunnel) cultivation for fresh produce is the most rapid crop management change that is currently taking place. Any changes in pest and disease levels inside plastic tunnels may be an indicator of wider effects that may be brought on by future climate change in N. Europe. The current arsenal of insecticides and acaricides is being severely limited by EU legislation. Some broad-spectrum products have already been withdrawn and others may only have a short life span in berry crops. The role of the remaining products in IPM programmes will be considered. Breeding for durable resistance to the major pests and diseases is one approach that should continue to be pursued. However, the long 'lead' time to introgress new sources of resistance using conventional breeding and the fruit industry's preference for 'quality' over pest and disease resistance traits may make this difficult to achieve in the short to medium term. However, the development of DNA marker-assisted breeding techniques should shorten the time before resistant cultivars with acceptable agronomic become available. In the interim, combinational strategies to extend the durability of IPM tools that are currently being developed must be found to fill in for any gaps in pesticide availability. As with all predictions, not all will come to fruition, but in Integrated Pest Management we should 'expect the unexpected'. Being forewarned may allow researches, extension workers and fruit managers to meet these challenges using flexible IPM programmes for raspberry pests.

Key words: *Byturus tomentosus,* pest management, pest prediction, protected cropping, raspberry aphids

Introduction:

Raspberry (*Rubus idaeus*) production in Europe is a relatively high value industry, some in the more remote mountain areas. In recent years there has been significant change in production in some parts of Europe, brought about by changes in the demand for different types of fruit or by attempts to exploit local advantages or to extend the growing season. Open-field production, mainly for processing fruit, remains the most widespread type of

production. However, in the west and southern Europe, use of protected (glasshouse) or semi-protected (polyethylene tunnels) growing conditions are now becoming the most important production systems providing high quality fruit for the fresh fruit sector. Historically, the majority of the raspberry industry in Europe has relied on pesticides to manage pests, diseases and weeds. Many of these pesticides were older products and as result of the recent review of pesticide usage in Europe, many such products e.g. malathion, dicofluanid, simazine are no longer available for use in this crop. The need to develop Integrated Pest Management (IPM) systems for raspberry has been recognised since the mid 1990s (Gordon et al. 1997a) and significant progress was made in Europe by the EU/BBW/industry funded Reduced Application of Pesticides in European Raspberry production (RACER) project (Gordon et al. 2002). Grassi et al. (2003) has shown that entomopathogenic nematodes can be used to manage larvae of *Otiorhynchus* spp. in the Trentino Region of Italy. In recent years there has been considerable success in managing the large raspberry aphid (*Amphorophora idaei*) by incorporating naturally occurring resistance genes into modern raspberry cultivars. However, in the UK the first resistant gene, gene A_1 has been largely overcome by resistant-breaking strains of the aphid and there is now evidence that the stronger source of resistance, gene A_{10}, derived from North American *Rubus occidentalis*, is being overcome by other resistant-breaking strains of *A. idaei* (Birch et al. 2003).

Drivers for change:

The main drivers for change in insect and mite management in *Rubus* production in Europe are 1) The EU-wide pesticide harmonisation regulations leading to the withdrawal of many pesticides (EU Pesticide Authorisation Directive 91/414/EEC) resulting in fewer products to control pests and diseases; 2) Supermarket and consumer demands for pesticide residue-free fruit (especially in the fresh sector) further driven by the 'healthy image' of soft fruits; 3) Changes in cultivation practices with increased use of protected and semi-protected cultivation and the use of long-cane plants to extend the growing season, restricting further the pesticides available for use under these growing systems. If these plants are not propagated under the high levels of plant health then pests and diseases may be introduced into the tunnels and this may result in rapid increased in damage; 4) the marketplace driven desire for quality often results in the more pest and disease susceptible cultivars being chosen for their yield and appearance rather than their intrinsic resistance to pest and diseases.

Insect and Mite Pest Management

Major pests – current control strategies

The major pests of raspberry in Europe are shown in Table 1. As an example of control strategies, the approved chemicals for both open-field and protected/semi-protected crops based on those approved at time of the conference are listed together with an assessment of their compatibility with possible integrated pest management systems (other products may be available elsewhere in Europe for control of these pests). In the case of aphids, natural plant resistance bred into current raspberry cultivars account for much of the control of the large raspberry aphid (*Amphorophora idaei*) but, at least in the UK, there is increasing evidence that the major source of resistance, gene A_{10}, is being overcoming in some raspberry cultivars (Birch et al. 2003). Two-spotted spider mite (*Tetranychus urticae*) has increased in importance in protected and semi-protected raspberry production in recent years. Growers have been faced with increased use of acaricides or use of predators (mainly Phytoseiid mites) and as a consequence a better understanding on the IPM compatibility of products is known.

Table 1. Major pests of raspberry and control products registered in the UK in open-field and protected/semi-protected cultivation (based on The UK Pesticide Guide – 2005).

Pest	Control strategies	Products	Open/Protected	UK registration	IPM compatible
Raspberry beetle	insecticide	chlorpyrifos	O	full	no
		deltamethrin	O	full	no
		rotenone	O/P	full	?
		thiacloprid†	O	SOLA*	possible
		bifenthrin††	O/P	SOLA*	no
Raspberry aphids	insecticide/ natural plant resistance	bifenthrin††	O/P	SOLA*	no
		chlorpyrifos	O	full	no
		pirimicarb	O	full	yes
		pymetrozine	O/P	SOLA*	yes
		rotenone	O/P	full	?
		nicotine	P	full	?
Raspberry cane midge	monitoring/ insecticides	chlorpyrifos	O	full	no
Two-spotted spider mite	acaricides/ predators	bifenthrin	O/P	SOLA*	no
		chlorpyrifos	O	full	no
		clofentezine	O/P	SOLA*	yes
		petroleum oil	O	full	yes
		tebufenpyrad	O	SOLA*	?

*SOLA – Specific Off-label Approval; † SOLA approval granted for use on berry crops during 2005 against capsids – incidental control for raspberry beetle and raspberry aphid. †† SOLA granted against wingless weevils - incidental control for raspberry beetle and raspberry aphid

Intermediate and minor pests – current control strategies

The intermediate pest list in Table 2 is based mainly on western European pest and may not fully reflect those found elsewhere but it shows that there is a heavy reliance on chemical application to manage these insects and mites. The increased use of entomopathogenic nematodes to manage wingless weevils, especially in warmer regions is encouraging.

Table 2. Intermediate level pests of raspberry in Europe and current control strategies.

Pest (common name)	Specific name	Control strategies
Raspberry leaf and bud mite	*Phyllocoptes gracilis*	Cultural/ cultivar selection
Wingless weevils	*Otiorhynchus sulcatus*	Chemical/ entomopathogenic nematodes
	O. singularis	Chemical
	O. armadillo	Chemical/ entomopathogenic nematodes
	O. ovatus	
Lepidoptera	*Lampronia rubiella*	Monitoring/ chemical
	Graphiphora augur	Monitoring/ chemical

Future developments

It is difficult to predict precisely what will happen in the future. However, as the number of available pesticides declines, combined with the changes in their use to meet the desire for very low levels of pesticide residues in the crop, the spectrum of insect and mite pests will change. The increase in temperature, either from climate change, or more likely, from the widespread adoption of semi-protected cropping for high-quality fresh fruit, will also allow some pests to increase in importance. Table 3 lists some of the known minor pest of raspberry in Europe. Some are known to be very localised pests, whilst others were considered pests in the pre-synthetic insecticide era before 1950.

Table 3. Minor pests of raspberry in Europe that may increase in importance with reduced pesticide use, increase in local temperature or by plant movement (list not complete).

Common name	Specific name	Pest status on raspberry	Pre-synthetic insecticides
Cantharid or Black soldier beetle	*Cantharis obscura*	minor (UK)	no
Loganberry cane fly	*Pegomya rubivora*	minor (Europe)	yes
Small raspberry sawfly	*Priophorus morio*	minor (UK)	no
Wingless weevil	*O. armadillo**	moderate (Italy)	no
Common green capsid	*Lygocoris pabulinus*	minor (Europe)	yes
European tarnished plant bug	Lygus sp.	minor (Europe)	no
Strawberry blossom weevil	*Anthonomus rubi*	minor (Europe)	no
Tortricid moths	e.g. *Cnephasia interjectana*	minor (Europe)	yes
Rosy rustic moth	*Hydraecia micacea*	minor	no
Flower chafer	*Oxythrea funestra*	minor (wild *Rubus* south Europe)	no

* *O. armadillo,* potential pest possibly spread by trade with Hardy Ornamental Nursery Stock (Barclay, 2003)

Three examples of minor pests that may cause concern

The three insects have been chosen to represent some of the potential problems that raspberry producers may have to face in the future as growing regimes change to adapt pressures applied to the industry.

The Cantharid or Black soldier beetle (*Cantharis obscura*) is a relatively recent pest of raspberry in eastern Scotland (Gordon & Woodford 1993), and damage attributed to this insect is still occurring in localised outbreaks. Adult beetles are attracted to the flowering laterals of summer fruiting raspberry in late spring and they chew and tear at the epidermis, causing the lateral to break. As *C. obscura* is one of several Cantharid beetle found on raspberry, and the only species that causes damage, it is essential that the grower accurately identifies which species is present before embarking on any chemical control programme.

Small raspberry sawfly (*P. morio*) is an insect that causes no significant damage to raspberry and blackberry in Europe, although some leaf feeding damage can be observed in open-field plantations late in the growing season. However, under protection, very large numbers can rapidly develop resulting in extensive defoliation of raspberry canes. With the

increase in protected and semi-protected cultivation there is a risk that damage caused by this insect will increase.

Several wingless weevils (*Otiorhynchus* spp.) are widespread in Europe and three have seen shown cause direct damage to raspberry. The vine weevil (*O. sulcatus*) and clay-coloured weevil (*O. singularis*) have been associated with damage in the UK, but concern has been expressed at the spread of another known pest of raspberry, *O. armadillo,* (Gordon et al. 2003), into Britain (Barclay 2003). Little is known about the biology of this insect and accurate identification of the insects requires specific knowledge of this group of beetles. It is possible that these insects have been spread unintentionally to other European countries as part of the Hardy Ornamental Nursery Stock trade. Should these weevils become established in local raspberry production areas, there is a potential that they could cause considerable damage.

Farm Practices

Implementation of newer technologies to meet the changing demands of the end-users of fruit produce will inevitably mean major changes in how the crop is managed and this will lead to increased demands on growers, their field staff, extension workers and advisors. This will require a period of continued training by appropriately qualified and experienced staff. Additionally it is likely that there will be a wider range of pests and diseases and, as correct and rapid identification will be the key to successful management, there is likely to be a greater demand for rapid diagnostic and laboratory facilities and development of in-field testing kits. Monitoring of pests and diseases to decide on appropriate management strategies by use of a range of techniques will increase. These will include local weather stations, use of various trapping systems including pheromone traps, natural plant volatile traps and coloured sticky traps to determine the thresholds of pests.

Pesticides

The range of pesticides available will inevitably decrease. It is highly probable that most, if not all, the organophosphorous-based insecticides will not be available for use in raspberry. Similarly, carbamate-based insecticides will be severely limited. Currently this leaves the synthetic pyrethroid-based products and several new molecules in development, or awaiting approval, as the main chemical control products. To be effective as part of an integrated pest and disease management system, these products will have to be fully tested to ensure they do not adversely affect any of the key non-insecticidal or microbiological biocontrol agents that may be developed as alternatives to current conventional control products. Effective soil sterilisation is a key to rapid replanting of may intensive small fruit crops. With the phased withdrawal of methyl bromide in Europe, suitable alternatives are being sought. The use of bio-fumigants is being tested in many centres and it is highly likely that some of the alternative systems will be applicable for use in fruit crops.

Semiochemicals

There is rapid development of semiochemical products for use against key raspberry pests. At SCRI, volatile flower attractants are being developed to attract both male and female raspberry beetles (Birch et al. 1996, 2002). Similarly, recent research at East Malling Research (EMR), in collaboration with researchers at Natural Resources Institute at the University of Greenwich, Chatham, has identified the female sex pheromone for raspberry cane midge (see Cross et al., this publication). Initially, both these attractant systems will be invaluable as part of an enhanced insect monitoring system to warn the growers that the insect numbers have exceeded that spay threshold, but further developments should lead to effective attract or lure and kill systems thus reducing the need to apply insecticides directly onto the crop. In a recent trial on enhanced monitoring in an unharvested blackberry plantation, the

white sticky traps with the floral attractant were approximately 50 times more effective than corresponding white sticky traps without attractant (see Figure 1).

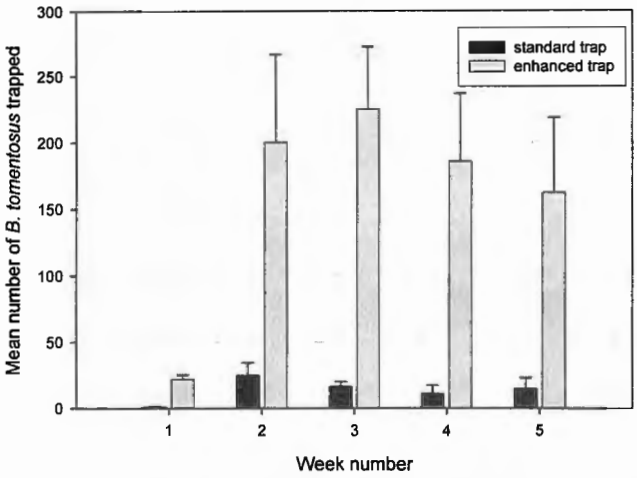

Figure 1. Mean number of raspberry beetles caught in the blackberry plantation at Norwich, England. Each experimental area contained two standard traps and two enhanced traps using flower volatile 'B'. Trapping started on 23 April 2003. Error bars represent standard error.

High health planting stock

Reduction in pesticides will lead to the greater use of high health planting stock from known high health sources. Growers who fail to do this put both their and their neighbour's plantations at risk. We foresee that there will be an increased use of pathogen testing services in Europe e.g. in the UK (www.fruithealth.co.uk). Similarly there will be an increase in the need for raspberry cultivars with higher levels of pest and disease resistance or tolerance. As breeding new cultivars is a long term strategy methods must be found to identify and permit the introgression of new resistance genes into the crop. Current research is underway to develop molecular markers to identify sources of resistance in raspberry (Graham et al. 2004, 2006).

Contaminants

Reduced pesticide usage and/or use of narrow-spectrum insecticides will increase the risk of fruit contamination by non-pest arthropod species such as looper caterpillars (Lepidoptera), predators (ladybird larvae (Coleoptera), spiders (Arachnids)), earwigs (*Forficula auricularia*, Dermaptera), and aphid parasitoids (Hymenoptera). Fruit contamination has been a problem in machine harvested open-field production systems in the UK and in North America where pre-harvest clean-up applications of low mammalian toxicity insecticides are applied (Gordon et al. 1997b). If contamination becomes a serious problem in fresh and high quality processed crops, additional applications of insecticides to the crop are likely and these may well negate any of the benefits from integrated pest management or low-input systems desired by the end users.

Conclusions and discussion

Raspberry production in Europe will continue to expand to meet the demands for both fresh and processed fruit as important components of a healthy diet. The industry will remain innovative, readily adopting new and improved production systems and cultivars. Many of the older pesticides will be no longer be available and there will be an overall decline in the number of products available, but newer, less environmentally damaging products will be introduced. These products will need to be fully compatible with the Integrated Pest and Disease Management (IPDM) systems that are evolving to meet the demands of end users for reduced pesticide residues in ripe fruit. It is highly likely that the demand for low, or even zero, detectible residues will extend to open-field produced fruit for processing as well as to fresh produce. Robust IPDM systems will develop throughout Europe, but it is highly likely that they will have to be 'tailored' for each individual region and may differ depending on the cultivar/s grown and their designated markets. Many of the currently important pests will remain but their relative importance will change as new cultivars, pesticide regimes and growing systems are introduced. Some minor or less important pest species will become more troublesome and it is probable that non-pest arthropods will increase in importance, often as fruit contaminants.

New technologies will be developed for control of several of the major pest species. The use of semiochemical-based monitoring and control is likely and the current developments with raspberry beetle floral attractants (Woodford et al. 2003) and raspberry cane midge sex pheromones (Cross, this volume) will lead the way. Soil pests and disease management will rely more on biocontrol strategies, such as green manures and biofumigation, increased use of entomopathogenic fungi and nematodes. The importance of high-health planting stock will increase as the arsenal of insecticides and fungicides diminish. Production of these plants will rely on stocks of high-health mother plants being held in secure, pest and disease-free conditions and well managed certification schemes, backed up with reliable and readily accessible diagnostic and laboratory facilities.

The introduction of these new technologies will require more complex crop management. To achieve this there will be an increased need for training and education of growers, fieldsmen and extension workers and supervisory staff.

Finally, there is an increased need for the industry to develop and adopt raspberry cultivars that have higher levels of pest and disease resistance than many of those currently preferred. In the short to medium term these pest and disease resistant cultivars may not have the high levels of 'shelf appeal' as the current susceptible varieties, but they may have greater benefits in reduced pesticide dependence.

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Further investigation of IPM methods for blackcurrant gall mite and leaf midge

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Abstract: Blackcurrant gall mite transmits reversion virus disease which causes sterility in blackcurrant bushes and is the main limiting factor in the life of blackcurrant plantations. Blackcurrant leaf midge attacks the shoots of blackcurrant, stunting growth. These pests have up till recently been controlled in commercial plantations in the UK by multiple sprays of the broad-spectrum pyrethroid insecticide and acaricide fenpropathrin (Meothrin) but this active substance is being withdrawn due to the EU pesticides review.

The results of three replicated field experiments are reported. In one, it was shown that combining the use of 1 or 2 early sulphur sprays with the use of varieties resistant to gall mite (e.g. Ben Hope) or to reversion virus (e.g. Ben Gairn) gives a very high degree of control of gall mite and reversion virus which is sustainable in the long term, and much better than either the resistant varieties or sulphur sprays alone. A standard treatment with Meothrin was shown to be at best only partially effective for gall mite control and much less effective than early season sulphur sprays. In the second experiment, sprays of sulphur were shown to be phytotoxic to blackcurrant causing leaf discolouration and significant loss in yield. Tebufenpyrad (Masai) is being investigated as an alternative to sulphur which can be used later in the season at times when sulphur is phytotoxic. The third ongoing experiment is investigating the effects of selective versus broad spectrum insecticide programmes on parasitism of blackcurrant leaf midge larvae by the parasitic wasp *Platygaster demades* to determine whether the parasitoid can be exploited to naturally regulate blackcurrant leaf midge. Only a very low incidence of parasitism was found in the first two years of the experiment.

Key words: acaricide, *Cecidophyopsis ribis Dasineura tetensi*, *Platygaster demades*, reversion virus, *Ribes* sp, sulphur, tebufenpyrad

Introduction

The results of three recent experiments at East Malling Research investigating IPM of blackcurrant gall mite and leaf midge are reported here. These experiments are a continuation of those reported by Cross & Harris (2004) and Cross & Harris (2003).

Combining host plant resistance and early sulphur sprays in IPM of gall mite and reversion virus

The gall mite and reversion virus experiment started in 2000 and the results of the first 3 years of which are described by Cross & Harris (2004) has been continued so that the long term effects of the treatments can be determined. The increase and spread of the virus over this period is of particular interest. Here we report the results of work done in 2003 and 2004.

The IPM experiment comprises a 5 x 5 Latin square of 25 plots. Each plot consists of 6 rows of 6 blackcurrant bushes; two rows of each of three varieties in a 3-way split plot design. The varieties are Ben Gairn (reversion resistant but gall mite susceptible), Ben Hope (gall mite resistant but reversion susceptible) and Ben Alder (susceptible to both gall mite and

reversion). Five different spray programmes were applied annually as treatments. Details of the treatments applied in 2003 and 2004 are shown in Table 1.

Table 1. Sprays applied for gall mite control and their dates of application.

Treatment	Growth stage (Gairn, Hope) and application date 2004				
	5 % emergence 1st leaf	Pre flower	50% emergence mid flower	End flower	+ 7 days
2003	26 March	25 April	30 April	15 May	29 May
2004	6 April	21 April	11 May	18 May	25 May
A	Sulphur				
B	Sulphur		Sulphur		
C	Sulphur		Masai		
D		Meothrin		Meothrin	Meothrin
E					
Dose rates: Meothrin (Fenpropathrin 100 g/l EC) - 0.5 l/ha; Masai (Tebufenpyrad 20% w/v WB) – 0.5 kg/ha; Sulphur 800 g/l SC – 12.5 l/ha					

Sprays were applied with an air assisted sprayer at a spray volume of 500 l/ha. The whole experimental area also received a programme of sprays of fungicides for mildew and leaf spot control. During early flower each bush of a variety was inspected for symptoms of infection by reversion virus disease this was 27 April 2004 for Ben Hope and Ben Gairn, and 13 May 2004 for Ben Alder the presence or absence of symptoms on each bush being recorded. On 8 December 2004, in the dormant period following the 2004 growing season, the blackcurrant gall mite galls on each bush were counted and recorded.

Overall numbers of galls increased considerably on all varieties indicating that both 2003 and 2004 were favourable years for gall mite migration (Table 2). The sulphur based spray programmes continued to give better control than the fenpropathrin programme. Ben Gairn and Ben Hope continue to show partial resistance to gall mite but the resistance is starting to be overwhelmed in the untreated plots. These results show the importance of using acaricide sprays on the resistant varieties to control gall mite. The resistance alone is not sufficient to control the gall mite.

The number of bushes with symptoms of reversion virus infection increased on the variety susceptible variety Ben Alder (Table2) which had the highest incidence of reversion virus. The highest incidence was on the untreated controls but the distribution was erratic and some virus infected bushes were found in all treatments. Lower levels of virus were present on the resistant varieties. Significantly, no virus infection was found on Ben Gairn, a reversion resistant variety, although a small number of infected bushes had been recorded the previous season. Numbers of reverted Ben Hope bushes did not increase significantly.

Table 2. End of season total number of blackcurrant gall mite galls per 60 bushes in the dormant period and total number of bushes per 60 bushes showing symptoms of reversion virus at the early flowering growth stage.

Treatment	Ben Alder (susceptible)		Ben Gairn (reversion resistant)		Ben Hope (gall mite resistant)	
	galls	revers	galls	revers	galls	revers
Year 4 (2003)						
A. 1 sulphur	22	2	1	1	4	6
B. 2 sulphur	24	5	15	3	0	0
C. 1 sulphur+ 1 tebufenpyrad	46	3	2	0	0	0
D. 3 fenpropathrin	282	2	66	0	54	2
E. untreated	748	6	382	0	103	0
Year 5 (2004)						
A. 1 sulphur	338	11	0	0	2	2
B. 2 sulphur	187	18	3	0	1	0
C. 1 sulphur+ 1 tebufenpyrad	701	14	2	0	0	2
D. 3 fenpropathrin	1694	17	294	0	266	2
E. untreated	7415	22	1381	0	472	1

The following conclusions can be drawn:

- Sulphur or sulphur then Masai spray programmes continued to give much better control of gall mite than the standard 3 Meothrin spray treatment. There was no difference between the single sulphur, the two sulphur or the one sulphur then one Masai treatments, which all gave a high standard of control.
- The resistant varieties Ben Gairn and Ben Hope continued to show high levels of resistance to gall mite, Ben Hope being more resistant than Ben Gairn. However, numbers of gall mite galls, increased by 3.6 and 4.6 fold from the end of the 2003 season to the end of the 2004 season on the untreated controls of the two varieties respectively, compared to a 9.9 fold increase on the untreated plots of the susceptible variety Ben Alder.
- Combining either of the resistant varieties with a sulphur or sulphur then Masai spray programme continued to give a very high standard of control of gall mite. Numbers of galls stayed the same or decreased for both varieties where these treatments were applied. The combination of resistant variety with a programme of Meothrin sprays was significantly less effective, gall numbers increasing 4.5 and 4.9 fold on the two varieties respectively.
- The incidence of reversion virus infection was greatest on the susceptible variety Ben Alder, especially on the untreated controls, the numbers of infected bushes increasing on average 5.2 fold. Numbers of infected bushes were too small to draw conclusions about the relative effects of the treatments in preventing reversion infection. The incidence of infection on Ben Hope stayed very low and even appeared to decline slightly and no reversion infection was found on Ben Gairn (reversion resistant). Interestingly, four Ben Gairn bushes were recorded as being infected in 2003 but the infection was not apparent in 2004.

Sulphur phytotoxicity experiment

The experiment was in a purpose planted blackcurrant plantation (planted with one year old rooted plants in March 2002) at East Malling Research. It comprised 960 blackcurrant bushes in 4 x 4 Latin squares, 2 squares of each of the five blackcurrant varieties (Baldwin, Ben Gairn, Ben Hope, Ben Lomond, Ben Tirran). Each Latin square consisted of 16 plots each of 6 blackcurrant bushes in a row. The rows spacing was 3.0 m with 0.5 m between bushes in the row and a 1.5 m spacing between plots.

Treatments (Table 3) were a factorial comparison of 4 sulphur spray treatments (pre flower, post flower, 3 sprays programme, untreated) on the five blackcurrant varieties. The sulphur an 800 g/l SC formulation 'Headland Sulphur' applied with a handlance at 12.5 l product in 500 l water/ha throughout.

Table 3. Sulphur spray treatments and the dates they were applied to each of the five varieties.

Treatment name	No of sprays	Growth stage of sprays	Dates of application (2003)		
			Ben Tirran	Baldwin	Other varieties
A. Pre-flower	1	Just before flower	15 May	16 Apr	16 Apr
B. Post flower	1	Just after flower	6 Jun	13 May	15 May
C. 3 spray prog.	3	Just before, just after flower + 2 weeks later	15 May, 6 Jun, 18 Jun	16 Apr, 15 May, 4 Jun	16 Apr, 15 May, 4 Jun
D. Untreated	0	-	-	-	

Assessments of the growth stage of each blackcurrant variety were made at 2-7 day intervals from 10 March - 8 June 2003 in order to determine the timing of sprays. The plots were inspected for visual signs of phytotoxicity on each occasion the growth stage assessments were made and careful inspections were made on 25 April, 3 June and 8 July 2003. Yields were recorded at harvest by hand picking and weighing the fruit from each bush in each plot separately. The fallen fruits from each plot were collected and weighed before picking was done. The fruit was harvested at the appropriate time for each variety, when the fruit was ripe. Shoot growth was assessed by measuring the total length of growth on the central bush in each plot on 9 March 2003 in the dormant period before treatments were applied and again on the same bush in the dormant period after the first year of the trial was completed on 9 January 2004. The length of the previous season's extension growth on each shoot was measured and the total for the bush calculated.

Analysis of variance was done on the yield. Square root transformation improved the analysis and t testing of mean log transformed values was done to determine which treatments significantly reduced yield at the $P \leq 0.05$ and $P \leq 0.01$ levels compared to the untreated for that variety. Analysis of variance was also done on the total length of extension growth per bush with covariance adjustment for the total length of extension growth before the treatments were applied.

Slight symptoms of phytotoxicity were apparent on 3 June 2003. The symptoms consisted of slight yellowing of the foliage which was most pronounced on the plots where the pre-blossom spray had been applied and on the plots where both the pre and post blossom spray had been applied at hat time. Symptoms were very slight on the plots that had received the post blossom spray only.

There were large statistically significant (P = 0.002) differences in the average yields of the different varieties. Ben Hope had by far the greatest mean yield (7.49 t/ha), Ben Gairn the smallest (2.89 t/ha) (Table 4). The ranking of the varieties in increasing order of yield was Ben Gairn < Baldwin < Ben Lomond < Ben Tirran < Ben Hope.

The sulphur spray treatment factor also had highly significant effects (P = 0.003). The programme of three sprays (treatment C) caused the greatest reduction in overall mean yield compared to the untreated control by 14.6% (Tables 4 & 5). The single just pre-blossom spray (treatment A) caused the second greatest reduction in the overall mean yield (by 11.4%) but the single post blossom spray treatment (treatment B) did not reduce the overall mean yield compared to the control significantly.

Caution has to exercised in interpreting the results for individual varieties because the interaction between the variety and the sulphur spray factors was not even nearly statistically significant (P= 0.366), even with the square root transformed data. The interaction between variety and spray treatment was not statistically significant (P = 0.108) even when comparing the mean if all 3 sulphur treatments with the untreated control in the square root transformed analysis. However, the greatest percentage reductions in yield occurred on the Baldwin treatments (Table 5) and this suggests that Baldwin may be more susceptible to sulphur than the other varieties though this is not proven by the statistical analyses.

The effect of the spray treatment and the interaction between variety and spray treatment on extension growth were not significant, or even nearly so, for any of these variates. However, the grand mean values of total shoot length for the spray treatments followed similar ranking and relative values to the ranking and relative values of the grand mean yield.

Table 4. Mean yield (ton/ha).

Variety	Pre flower A	Post flower B	3 spray prog C	Untreated	***Mean***
Gairn	2.85	3.11	2.59	3.02	**2.89**
Lomond	5.96	6.19	5.69	6.01	**5.96**
Hope	7.06	7.81	6.71	8.40	**7.49**
Baldwin	4.49	4.53	3.68	5.58	**4.57**
Tirran	5.80	7.21	6.52	6.50	**6.51**
Mean	**5.23**	**5.77**	**5.04**	**5.90**	**5.48**

	Fprob	SED (75 df)
variety	0.002	0.422
spray	0.003	0.259
variety . spray	0.366	0.655

Table 5. Percentage increase (positive values) or percentage decrease (negative values) in mean yield compared to the untreated control.

Variety	Pre flower A	Post flower B	3 spray prog C
Gairn	-5.6	+3.0	-14.2**
Lomond	-0.8	+3.9	-5.3*
Hope	-16.0**	-7.0*	-20.1**
Baldwin	-19.5**	-18.8**	-34.1**
Tirran	-10.8	+10.9	+ 0.3
Mean	**-11.4****	**-0.2**	**-14.6****

Significantly less than the control for that variety * $P \leq 0.05$, ** $P \leq 0.01$

The following conclusions were drawn:

- A single foliar spray of sulphur applied just before flowering was phytotoxic to 2 year old bushes of the blackcurrant varieties Ben Gairn, Ben Hope, Ben Lomond, Baldwin and Ben Tirran causing leaf discoloration, an 11.4% reduction in yield and possible slight reductions in growth.
- A single spray of sulphur just after flower did not significantly reduce yield or growth.
- A programme of 3 sprays, one just pre-flowering, one post flowering and a third approximately 14 days later, caused greater phytotoxicity than the single pre-flowering spray, reducing yield on average by 14.6%.
- The data suggests that Baldwin may be more sensitive to sulphur than the other varieties, but this could not be proven by detailed statistical analyses.

Gall mite and leaf midge IPM experiment

A new blackcurrant gall mite and leaf midge IPM experiment was established in an existing mature commercial blackcurrant plantation at Upper Horton farm, Canterbury, Kent. The plantation cv Ben Lomond, approximately 2.3 ha in area was divided into 12 plots of approximately equal size in a 4 x 3 array. Each plot was 6-8 rows wide and 80-90 m long for the purposes of the experiment. The experiment compared three treatments (Table 6), an IPM treatment using selective insecticides and acaricides only, a conventional treatment using broad spectrum products and an untreated control.

Sprays were applied at a volume of 500 l/ha with the growers Commandair orchard airblast sprayer. The whole experimental area also received a programme of sprays of fungicides for mildew and leaf spot control as follows: The number of leaf midge galls in shoots was counted and a sample of galls from each plot was collected and reared to adult to determine the % parasitism. Large numbers of galls occurred on all plots but a very small percentage (< 1% in 2004, < 2% in 2004) of larvae were parasitised by *Platygaster demades* (Table 7). Emergence observations were terminated after the 10 June 2003 even though only < 65% of larvae had emerged either as an adult leaf midge or as a parasitoid. This work does show that the parasite does occur in the experimental site, though parasitism levels were small. Introduction is thus unnecessary.

Table 6. Treatments in the gall mite and leaf midge IPM experiment at Upper Horton farm.

Conventional treatment	IPM treatment	Untreated
• Sulphur at bud break • Sulphur at first grape emerged • Dursban pre flower • Meothrin just pre flower • Meothrin end of flower	• Sulphur at predicted first emergence of gall mite • Aphox pre-flower • Sulphur or Masai at peak emergence	• No insecticides or acarcides

Table 7. Parasitism of leaf midge larvae sampled on 30 April 2003 by the parasitoid *Platygaster demades*.

Treatment	Total number of larvae reared	Total number of leaf midge adults emerged	Total number of *Platygaster demades* adults emerged
2003			
IPM	400	227	2
Conventional	400	250	1
Untreated	400	205	1
2004			
IPM	400	292	5
Conventional	400	307	9
Untreated	400	286	6

A small percentage of first-generation leaf midge larvae (< 2%) were found to be parasitised by the parasitoid *Platygaster demades*. Although the rate of parasitism has increased since 2003, it is not sufficient to significantly affect leaf midge populations. The lack of growing shoots to support second and third generation midge attacks due to the fruit load being carried by the bushes may be depriving *P. demades* of its host at crucial periods, so preventing more rapid establishment.

Conclusions

Some useful conclusions can be drawn from the results of these three experiments. It was shown that combining the use of 1 or 2 early sulphur sprays with the use of varieties resistant to gall mite (e.g. Ben Hope) or to reversion virus (e.g. Ben Gairn) gives a very high degree of control of gall mite and reversion virus which is sustainable in the long term, and much better than either the resistant varieties or sulphur sprays alone. The standard Meothrin treatment was shown to be at best only partially effective for gall mite control and much less effective than early season sulphur sprays. In the second experiment, sprays of sulphur were shown to be phytotoxic to blackcurrant causing leaf discolouration and significant loss in yield.

Tebufenpyrad (Masai) is being investigated as an alternative to sulphur which can be used later in the season at times when sulphur is phytotoxic. The third experiment is investigating the effects of selective versus broad spectrum insecticide programmes on parasitism of blackcurrant leaf midge larvae by the parasitic wasp *Platygaster demades* to determine whether the parasitoid can be exploited to naturally regulate blackcurrant leaf midge. Only a very low incidence of parasitism was found in the first two years of the experiment and further work is needed to determine whether parasitism levels will increase in the longer term.

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