

Session 11A

Developments in Crop Protection, including IPM Strategies, in Modern Horticultural Crop Production Systems 2

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Assurance schemes: a route for research into practice?

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Introduction

The Assured Food Standards Assured Produce Scheme was established in 1997 as a retailer-producer partnership to deliver improved food safety and traceability through a set of auditable standards based on the principles of good agricultural practice. Processors and retailers prefer produce to be grown according to the audited standards, so compliance assists access to markets. Such crops now account for approximately 75% of output of fruit, vegetables and potatoes in the UK to a value in excess of £1 billion. Increasingly, accreditation to the Assured Produce Scheme (APS) is also being specified in public sector procurement contracts for the supply of fresh and frozen produce to Government Departments including the National Health Service and to the 'Schools Fruit and Vegetable Scheme' for primary school children in the UK. The consequence is that consumers buying Assured Produce can be confident in the safety and integrity of the produce they eat.

History

The Food Safety Act (1990) in the UK emphasised the need for retailers to ensure the safety and traceability of the food they sell. This need for due diligence resulted in the development of schemes that provided assurance of the quality of fresh produce through the whole supply chain. The history of the development of the Assured Produce Scheme has been set out in detail by Payne (2004). Briefly, the NFU-Retailer Partnership was established in 1991 between most of the UK multiple retailers and the National Farmers Union (NFU). Crop production standards were established that covered legal compliance and good agricultural practice that would deliver the due diligence required for food safety. Due to the great diversity of crops and their associated production systems, individuals with considerable technical experience of each crop developed specific protocols for each crop type. Once crop production protocols had been established, a mechanism was required to audit the standards to ensure compliance. Independent auditors were appointed to ensure compliance on the farm. Initially audits were done once every three years but are now annual. In 2001, the Scheme standards were fully accredited to the European EN45011 standard by the United Kingdom Accreditation Service (UKAS).

The original aim of APS was to develop a common set of standards to which all growers would adhere enabling them to market their produce to all retailers. However, from the outset some retailers have required their point of difference and have developed their own parallel schemes. These include 'Nature's Choice' (Tesco), 'Field to Fork' (Marks and Spencer) and LEAF Marque (Waitrose), though each has APS as their core standards.

Retailers also deliver to consumers supplies of fresh produce through imports. The due diligence for imported produce is delivered through the international fruit and vegetables assurance scheme EurepGAP (European Retailer Protocol Good Agricultural Practice) which is supported by UK retailers. The APS standards have been benchmarked against EurepGAP since 2002 to avoid the divergence of schemes, though the converse does not apply.

In 2000, APS was a founder member of Assured Food Standards (AFS) which brought together a number of assurance schemes that individually managed production standards for cereals and oilseed rape, beef, lamb, pigs, chickens and dairy produce. AFS licences the 'Red Tractor' logo on products marketed through supermarkets.

The standards

The standards are based on the spread of best practice to deliver good agricultural practice and have their foundations in the principles of integrated crop management. The Generic Protocol Standards are reviewed continually by a Technical Advisory Committee, and are supported by the Generic Protocol Guidance Notes. The Generic Standards are supplemented by some 50 crop specific protocols that are reviewed annually by specialist crop authors with detailed knowledge and experience of the individual crops. These latter documents provide considerable technical detail on the production of each crop. The Generic and Crop specific protocols are available on the APS website (www.assuredproduce.co.uk). The Standards contain control points against which the production process is independently audited.

New research findings

Much research and development in agriculture and horticulture is incremental. The accumulation of these incremental developments over recent decades has resulted in substantial improvements in productivity and quality. The incorporation of new research findings, once proven, into best practice can be achieved rapidly through inclusion into individual crop protocols. The authors of the individual crop protocols are key to this process through their knowledge of current research and development findings, and how they relate to the production of their crop. At this stage new research findings are included to aid the rapid spread of research findings to producers. Should individual producers, or their advisors, require access to original research reports, they are available through the ADLib electronic library available to members via hyperlinks from the APS website. These include the output of research funded by the Horticultural Development Council, British Potato Council and many others.

The APS also does its own development work where it is relevant to its main objective of delivering safe food. This is well illustrated by work done to further minimise pesticide residues on fresh produce at the point of sale and to advocate non-chemical methods of pest control (Payne & Gibbard, 2005). Recently a questionnaire to growers has demonstrated that a significant majority of growers had modified their use of pesticides to further minimize residues. Particular progress has been made in refining the use of CIBC (chlorpropham) as a sprout suppressant on potatoes in store to reduce residues further. Recently work has begun to understand better the potential risks of the microbiological contamination of produce from the use of irrigation water and how the risks might be minimised, particularly on produce eaten without further processing. Findings will be implemented through the application and auditing of revised standards.

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Status of IPM, pesticide use and misuse, and information transfer in horticultural crops in Albania, Ukraine and Moldova: participatory appraisal and baseline survey for tomato, cucumber, grape and apple

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The Integrated Pest Management Collaborative Research Support Program (IPM CRSP) is an international IPM effort funded by USAID and managed by Virginia Tech. The goal is to develop appropriate IPM technology and foster its implementation in various developing countries and transitional economies. Eastern Europe is one of several CRSP regional projects around the globe. In the first phase of the Eastern European regional project, a multidisciplinary team focused on a single crop, olive, in Albania (Pfeiffer *et al.*, 2005). A second phase of the IPM CRSP has now been initiated. In the new phase, the Eastern European regional project has expanded both geographically and agriculturally. The countries involved are now Albania, Ukraine and Moldova. The research focus now involves tomato, cucumber, grape and apple, crops that are common to all three countries.

Participatory appraisal

The first step in determining an appropriate research plan was a Participatory Appraisal (PA). The aim was to determine the primary pest management issues that need to be addressed, and, furthermore, the main obstacles to their resolution. To perform the PA, a small group of US scientists helped form a larger appraisal team with host country scientists. The PA for the new crops in Albania was held in August 2004; for Ukraine and Moldova in May 2006. Each PA team included four Americans and interdisciplinary teams of host country specialists. The PA revealed knowledge gaps among growers, concerns over pesticide safety and quality, and lack of extension infrastructure. Many pesticides are applied without regard to monitoring, pest biology or safety. Many pests are uncontrolled, reducing farm profitability. These problems must be overcome to meet goals of reduced pesticide use while providing high value produce for domestic and international markets. Problems common among sites relate to privatization following collapse of a centralized economy, with a generation of farmers lacking experience in farm decision-making. Former large-scale collective farms have been divided into smaller units and are under management of individuals with little decision-making experience. This is exacerbated by

problems in other sectors resulting in people moving from other occupations (engineering, music, construction etc.) into agriculture with the goal of greater profitability. Some grower concerns need to be addressed. For example, some felt there was a problem with pesticide merchants selling adulterated pesticides, resulting in control failures. While this may be the case, growers often had a poor understanding of pest phenology, and are moving from older, broad spectrum, materials like dimethoate to newer, more selective, materials like neonicotinoids. With a lack of proper application techniques, control failures could also result.

Baseline survey

As a second step, early in the project and following the PA, a Baseline Survey was conducted (summer-fall 2006). This approach employs a more structured survey tool, where a greater number of farmers were interviewed in order to obtain a more quantitative assessment of the pest management situation at the outset of the project. The objective is to allow a future determination of the impacts of our research and technology transfer efforts. Sixty families were targeted, and asked a standardized set of 113 questions that addressed knowledge of pests, pest management concerns, size of farm families, division of farm decision-making, and farm economics. Using Moldova as an example, about a third have planted new orchards, vineyards or built new greenhouses since privatization. About 42, 54, 48, and 0% applied insecticides in tomato, cucumber, apple and grape, respectively, whereas 73, 88, 37, and 96% applied fungicides. Price and specialists advice were almost equally regarded as the most important factors (very important) in making pesticide selections, whereas 18% thought pesticide dealers' advice somewhat important. About 43% attributed health problems for them or their family to pesticides. The three most severe tomato pests are phytophthora, *Heliothis armigera*, and Colorado potato beetle. The three most severe cucumber pests are peronospora, mildew, and aphids. The three most severe grape pests are mildew, oidium and grape berry moth. The three most severe apple pests are codling moth, apple scab and mildew. When asked if applying pesticides would increase yield, 90% replied yes. When asked if applying pesticides could stimulate a pest infestation, 65% said yes. The percentages applying pesticides in each crop were: *tomato*: fungicides 85%, insecticides 64%, herbicides 18%; *cucumber*: fungicides 88%, insecticides 50%, herbicides 25%; *grapes*: fungicides 100%, insecticides 25%, herbicides 0%; *apple*: insecticides 51%, fungicides 46%, herbicides: 9%. Only 17% had attended training sessions in the last 5 years, though 70% would be interested. When asked if an agricultural technician, who discussed non-pesticide means of controlling crop pests, ever visited the farm, 67% replied no. Almost half of respondents felt they did not have access to adequate information. In each country, a subset of farms was selected where both the husband and wife were interviewed. This will allow a gender analysis of IPM knowledge and practice for the project. This survey will not only enable more quantitative descriptions of the state of IPM, but will allow measurement of progress in research and extension during the project.

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Progress towards industry-wide sustainable IPM in Florida's strawberries

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Strawberry production in Florida, USA has increased from about 1,600 ha, valued at about \$65 million, in 1977-1978 to about 3,200 ha, valued at about \$210 million, in 2006-2007. The entire production is for fresh-market winter sales along the USA east coast. The crop is grown using plastic mulch, and an annual hill system of culture from 50,000 bare-root transplants per ha dug from northern or high altitude regions and planted mostly during October. Fruits are harvested from late November until April when prices fall as more abundant California berries are shipped eastward, but peak production occurs in January and February. Almost the entire crop is grown in west-central Florida near Plant City, at about 28°N latitude.

The sub-tropical climate presents an environment favorable for numerous nematode, weed, microbial, and arthropod pests. Fungal diseases are the most destructive pests of the crop in Florida but arthropods are also a considerable threat and require effective management. The principal arthropod pests have not changed in the past three decades and include the principal pest, two spotted spider mite (*Tetranychus urticae*), and also southern and fall armyworms (*Spodoptera eridania* and *S. frugiperda*), flower thrips (*Frankliniella bispinosa*), melon aphid (*Aphis gossypii*), a *Drosophila* spp. fruit fly and three or more species of sap beetle (Nitidulidae), although several other arthropods occasionally reach economic pest status.

These pests were controlled, in the most part, prior to 1978 with broad-spectrum pesticides including organophosphates, carbamates, and chlorinated hydrocarbons applied on a regular basis, and with little regard for the ecological status of the arthropod community. About that time, scouting to assess the ecological condition of the strawberry fields was introduced and pesticide use began to be determined accordingly.

By the 1990s, *Phytoseiulus persimilis* predators were being introduced on some farms for control of spider mites, along with reduced use of ecologically disruptive pesticides. As more farmers adopted the practice over the next few years, a new awareness emerged of the ecological damage in the crop from broad-spectrum pesticides. New pesticides including several biologically derived products, with better target-pest specificity and other favorable environmental qualities, replaced many of the more harsh early pesticides.

The following, generally environmentally harsh, insecticides have been removed from use by Florida strawberry farmers: methoxychlor, toxaphene, azinphosmethyl, carbophention, demeton, ethion, fonofos, methyl parathion, mevinphos, oxydemeton methyl, parathion, cryolite, and cyhexatin. Only a few environmentally damaging ones remain.

Today many Florida strawberry farmers rely on biologically and ecologically based plans of arthropod pest management and are seeking new biological controls for aphids, thrips and other pests in addition to spider mites.

The strawberry industry in Florida has expanded greatly over the past 30 years and represents a unique example of an annual, field-grown, horticultural crop produced with an applied biological component of pest management. There has been an intensive effort during the past three years to deliver programs of biological control for pests other than spider mites to Florida's strawberry production. Success in that respect is likely to continue. At the close of three decades of progress toward sustainable management of arthropod pests in Florida strawberries, a system of protection exists that is completely different from the one used at the opening of the period. A strong foothold of sustainability has developed.

The overwintering chasmothecia of *Podosphaera aphanis* and the initiation of the subsequent epidemic

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Commercial strawberry production in the UK has used polythene (Spanish) tunnels since 1993. This protection was introduced to give a harvest period of five months rather than the six weeks from unprotected strawberries. The covering protects the plants from rain damage which was the main agent for the spread of grey mould fungus, *Botrytis cinerea*. Polythene tunnels have thus improved the conditions for strawberry production and controlled grey mould. The environment created in the polythene tunnels is, however, favourable for the growth and development of powdery mildew of strawberries, caused by *Podosphaera aphanis*. Epidemics of *P. aphanis* are now the main threat to strawberry production in the UK. Strawberry breeders in the UK concentrate on fruit quality and appearance. As a consequence only a narrow range of varieties is grown, most of which are disease susceptible (Dodgson *et al.*, 2005). Growers could manipulate the tunnel environment to control *P. aphanis* but in practice the conditions required to produce high quality fruit correspond to the conditions that enhance epidemics of *P. aphanis*. Disease control in commercial crops is, therefore, dependant on regular applications of fungicides.

Strawberry plants remain in the ground, and produce crops, for three or four seasons. The plants are uncovered throughout the winter, and covered with polythene tunnels from spring to autumn. Thus plants are exposed to ambient conditions in the winter (temperature, humidity and precipitation) and artificial conditions throughout the growing and harvesting period. Temperatures of 15 to 30°C and humidity over 95% represent ideal conditions for epidemics of *P. aphanis* (Dodgson *et al.*, 2005).

In 2005 it was reported (Dodgson *et al.*, 2005) that *P. aphanis* was overwintering on strawberry plants between seasons. The work reported here aimed to track the disease on plants in the field from the removal of the tunnel covering in the autumn through to the replacement of the tunnels in the spring and the subsequent build up of the epidemic.

Materials and methods

Green and necrotic leaves were sampled at monthly intervals from November to June. The area sampled had shown disease the previous summer, and 50 to 150 leaves of each leaf type were sampled at each date. All leaves collected were scanned under a dissection microscope and those with chasmothecia noted and further examined under the light microscope at x100. The width and length ratio of 50 chasmothecia, the number of chasmothecia containing asci, and the presence of ascospores were all recorded. In April, after the crops had been covered, symptom of *P. aphanis* developed and microscopic examination of the developing colonies was carried out. The numbers of colonies arising from ascospores was noted and the length/width ratio of the ascospores was calculated.

Results

Chasmothecia only occurred on a small proportion of sampled leaves (Table 1) but they occurred throughout the winter and were most common on necrotic leaves. The size of the chasmothecia was variable. In December, March, April and May, the majority of

chasmothecia contained asci. In June no chasmothecia were found on any of the 200 leaves examined. In early April, all colonies, and in late April 97% of colonies, examined grew from an ascospore (Table 2). In May and June this dropped to 88% and 86%, respectively.

Table 1. Size of chasmothecia, and number containing asci and ascospores (GL = green leaves, NL = necrotic leaves)

Sample date	Percentage of leaves with chasmothecia		Length/width ratio of 50 chasmothecia		Number of chasmothecia with asci		Number of chasmothecia with ascospores	
	GL	NL	GL	NL	GL	NL	GL	NL
7/11/06	16.7	0	1.03	0	22	0	28	0
5/12/06	1.7	5.1	1.03	1.02	44	43	6	7
10/01/07	0	1.9	0	1.03	0	22	0	28
7/02/07	0.4	3.3	1.03	1.03	24	15	26	35
13/03/07	0.7	1.9	1.02	1.02	0	48	0	2
11/04/07	0	1.9	0	1.01	0	50	0	0
14/04/07	0	2.4	0	1.03	0	44	0	6
18/05/07	0	0.8	0	1.02	0	47	0	3
7/06/07	0	0	0	0	0	0	0	0

Table 2. Colonies originating from ascospores

Sample date	Number of colonies	Number of colonies without an ascospore	Percentage of colonies without an ascospore (%)	Length/width ratio of the ascospores (number measured)
11/04/07	18	0	0.0	1.13 (18)
19/04/07	45	1	2.2	1.19 (44)
03/05/07	77	1	1.3	1.18 (76)
18/05/07	74	16	21.6	1.20 (58)
07/06/07	38	5	13.2	1.10 (33)

Discussion

Though growers and agronomists have been aware of the presence of overwintering structures (chasmothecia) of *P. aphanis*, the significance of them in the initiation of epidemics had not been appreciated. Growers concentrated on the control of *P. aphanis* after symptoms were visible. The work reported here shows that the chasmothecia develop and survive in the winter and that the ascospores initiate new infections in the spring after conducive conditions develop in the tunnels. This has important consequences for disease management as fungicides could be used to reduce this initial inoculum in the early spring. Further investigation of the environmental conditions that influence the chasmothecia in winter could be incorporated into the predictive model under development.

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Differential effects of gibberellic acid on the growth of *Botrytis cinerea* isolated from various ornamental plants

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Various hormones are involved in the growth of *Botrytis cinerea* Pers.: Fr., and they are generally the same as those found in plants. It has been shown that this fungus produces ethylene and indolacetic acid but, so far, gibberellic acid (GA₃) and abscisic acid production in *Botrytis* spp. has not been proved unequivocally (Sharon *et al.*, 2004). Although hormones play an essential role in fungal growth, further studies are necessary to understand the pathways of phytohormone biosynthesis in *Botrytis* spp., their effects on fungal growth, and their interactions with plant growth. GA₃ is a commonly occurring hormone found in plants and some fungi, including *Gibberella fujikuroi* and *Sphaceloma manihoticola*. GA₃ promotes cell division and/or cell elongation in plants, and is relatively abundant in juvenile plant tissues. Exogenous application of GA₃ to plants can reduce their susceptibility to infection by *Botrytis* but, rarely, it has been shown to promote disease (Sharon *et al.*, 2004).

The genus *Botrytis* has a thallus constructed from filamentous hyaline and pigmented or brown-striped hyphae. Conidiophores or hyphae bearing conidia arise from the hyaline or pigmented hyphae. The apical cell of the conidiophore are enlarged or rounded, bearing clusters of conidia. The conidia are hyaline or grey in mass, one-celled and ovoid. Most species of *Botrytis* are aggressive saprophytes, which also invade injured plant tissues causing Botrytis blight (grey mould) which is common in ornamental plants. Sclerotia are firm aggregations of vegetative hyphae with determinate growth. They contain reserve materials and after maturity are capable of independent persistence for a considerable period of time. Sclerotia formation is a complex process which involves both intrinsic and extrinsic factors. Formation depends on appropriate environmental factors, such as temperature and light, which do not necessarily coincide with those favourable for growth.

In the present work, the effects of exogenous applications of GA₃ on the growth of *B. cinerea* *in vitro* have been studied. *B. cinerea* was isolated from various ornamental plants including *Lantana camara*, *Lonicera japonica*, *Hydrangea macrophylla*, and *Cyclamen persicum* affected by Botrytis blight in Southeast Spain. Fungi isolated from these plants were identified as *B. cinerea* using polymerase chain reaction (PCR) with a molecular marker specific to this species (750 bp), which is present in all *B. cinerea* strains and absent from other species of *Botrytis*. The fungi were isolated and purified on potato dextrose agar PDA (Scharlab, Barcelona, Spain). These pure isolates were cold stored until experiments were set up the following day. Four GA₃ solutions containing 0, 10, 100 or 1000 mg/100 mL were prepared in sterilized distilled water from GA₃ 90% p/p Fluka, Buchs, Switzerland. Four replicates were prepared per GA₃ concentration and isolate. Each Petri dish was filled with 1 mL of GA₃ solution to which 19 mL of molten PDA was added to obtain 0, 5, 50, and 500 mg/L. Petri dishes were refrigerated until the following day, when a small disk (3-mm diameter), cut from actively growing pure cultures of *B. cinerea* on PDA, was placed onto the centre of each plate, fungal side downward. Dishes were incubated at 26°C in normal daylight conditions until sclerotia had developed and matured. Several

variables were measured such as mycelial growth, conidiation, conidial dimensions and sclerotial development. ANOVA was applied and means were separated by LSD intervals.

Vegetative mycelium was variable among the different isolates. *B. cinerea* isolated from *L. camara* showed a typical grey mycelium with clear conidiation occurring from seven days. The isolates from *C. persicum* were similar but with less overall conidiation. Initially, this occurred in the centre of the colony but from eight days had spread across the entire surface of the colony. However, isolates from *H. macrophylla* and *L. japonica* showed a more white than grey mycelium after five days at 26°C. Only the isolate from *L. japonica* showed a similar grey aspect after 10 days. Generally, GA₃ had no effect on the appearance of colonies, except for the isolate from *L. camara*, in which conidiation was less on the young mycelium grown with 500 mg/L. GA₃ had some influence on both mycelial growth and conidiation. Conidiation of the isolate from *H. macrophylla* only began after 18 days with the five and 50 mg/L GA₃ applications. Growth rate was calculated from the difference in colony diameter measured between the third and fourth days of culture at 26°C. Five mg/L of GA₃ slightly increased the growth rate of *B. cinerea* isolated from *H. macrophylla* and *C. persicum*. *B. cinerea* isolated from *L. camara* grew more rapidly than that isolated from *H. macrophylla* and *C. persicum*. The overall growth rates were: 1.60 (*L. camara*), 1.24 (*L. japonica*), 1.26 (*H. macrophylla*), 1.19 (*C. persicum*) cm/day. Sharon *et al.* (2004) reported that GA₃ increased mycelial growth and conidiation *in vitro*, at concentrations of 1.0 and 50.0 µg/L, respectively. However, this has not been confirmed by all researchers. GA₃ had no effect on conidial size, but mature conidia of isolates from *L. camara* and *L. japonica* were less than 15 µm in length, whereas those from other isolates were larger, reaching 18 µm. The number of sclerotia developed was significantly affected. 5 mg/L of GA₃ promoted sclerotia formation in the isolate from *H. macrophylla* but did not alter sclerotia dimensions. Martinez *et al.* (2007) reported that exogenous application of 1 mM GA₃ (346 mg/L) as a spray when vegetative mycelial growth had reached a diameter of ≥6 cm and before conidiation had occurred, increased the number of sclerotia of *B. cinerea* isolated from *Chamelaucium uncinatum* at the mature stages of fungal growth.

These results imply that several isolates of *B. cinerea*, especially from *H. macrophylla*, were affected by GA₃. The least vigorous isolates were the most affected by GA₃. It is possible that some isolates, probably the most vigorous, synthesize this hormone. However, although a gene (*bccps/ksI*) showing significant homology to the first gene of the GA₃ pathway in *G. fujikuroi* has been identified in *B. cinerea*, it seems that expression of this gene could not be detected under any growth conditions (Sharon *et al.*, 2004).

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Effect of *Phytophthora* spp. on the growth of tomato plants treated with *Pseudomonas oryziphilans*

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With increasing public awareness of the environmental implications of the overuse of chemical pesticides in agriculture, alternative strategies for the control of plant diseases are being sought (Weller, 1988). The nematode symbiotic bacterium *Pseudomonas oryziphilans* has been shown to be effective in field trials for the suppression of a number of fungal diseases of seedlings. *P. oryziphilans*, which also shows antibiotic activities against several pathogens *in vitro*, was investigated for its ability to suppress damping-off and root-rot of tomato seedlings caused by *Phytophthora megasperma* and *Phytophthora cryptogea*. Greenhouse studies showed that application of bacteria gave effective control of *P. megasperma* and *P. cryptogea*, the causative agents of damping-off disease. Seed treatments with *P. oryziphilans* not only increased seedling stand, plant height and fresh weight but also decreased root rot severity compared with untreated seeds in pathogen-infected soil. To provide more insight into the role of *P. oryziphilans*, two treatments were tested a) soil drenching and b) seed coating, at four bacterial densities.

P. oryziphilans was obtained from the haemolymph of *Galleria mellonella*, which had been infected using infective nematode juveniles of *Steinernema abbasi*, and was cultured on Nutrient Agar (NA). A pure colony was cultured in nutrient broth (30g/L) and clear bacterial suspensions were taken as described before (Kapsalis *et al.*, 2003). *P. megasperma* and *P. cryptogea*, which were obtained from the University of Reading culture collection, were maintained on Potato Dextrose Agar (PDA) and were stored in the dark at 15°C.

Seeds of the tomato cultivar Carabola were planted in plastic pots (size 8 x 7 cm) that had been dipped in 70% alcohol for 24h. One seed per pot was sown into a traditional loam based compost (John Innes No 2). Greenhouse temperature was approximately 25-27°C. The potential of the biocontrol agent used as seed treatment ('seed coating') and as a soil drench to reduce the incidence and severity of disease on plants was evaluated. Seeds may be colonized by the bioprotectant before planting. This colonization permits the bioprotectant to be positioned before the seed is exposed to either competitive microflora or plant-pathogenic fungi (Harman, 1991). Four suspension concentrations of 10⁶ to 10³ cells per ml of the *P. oryziphilans* were tested. Bacteria were inoculated either by applying 4ml of the bacterium suspension directly as a soil drench into pots, or by soaking seeds in the suspension for 10 min. After planting the seeds, the soil was kept moist as necessary. The height and the weight of the plants were measured after 40 days. Randomised designs were used to test the two pathogens, four concentrations of the bacterium and two different ways of applying the bacterium suspension. Experiments were repeated three times with four replications per treatment.

Plants treated with *P. oryzihabitans* had significantly reduced disease incidence and higher plant weights compared with the pathogen control soil in the *in vivo* experiments. Soil drenching treatments using higher bacterial populations (10^6 cells/ml) resulted in significant differences in plant weight especially in soil infested by *P. megasperma*. In *P. cryptogea*-infested soil, soil drenching concentrations of 10^3 cells/ml of *P. oryzihabitans* significantly increased plant weight and showed that even smaller number of bacterial cells are capable of disease suppression.

The most practical methods of applying bacterial inocula in the field are seed-coating or spraying of bacterial suspensions directly onto the soil. However, in all cases investigated in the development of the seedling assay, disease control was most effective when the bacterium was applied as a soil drench prior to sowing. These results show that of the two ways of applying *P. oryzihabitans* tested in the current study, soil drenching was more promising than seed coating. This implies that the initial interactions in the soil were significant for disease suppression, where prevention of oospore germination is the key. Although the incidence of disease was dependant on the relative numbers of pathogen propagules in the soil, and especially in the soil infected with *P. megasperma*, the actual number required for significant disease occurrence was low.

It is apparent that *P. oryzihabitans* is multifactorial in its mode of action for the control of *P. megasperma*- and *P. cryptogea*- induced damping-off disease of tomato. The bacterium had the capacity to inhibit germination of pathogen oospores, its growth and the infection process. By determining the ecological interaction between the host, pathogen and putative biological control agent, the efficacy of disease suppression can be maximized under field conditions. For example, considering the apparent mode of action, the timing and frequency of inoculum application need to be made to coincide with the local environmental conditions under which control will be successful.

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Apple: integrating pest and disease forecasting and management in India

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In the Indian Himalayas, apple crops are grown throughout the temperate region and cultivation extends from the Northwest to the Northeastern hills, occupying a prime position among various temperate fruits and accounting for 55% of the total area and 75% of total production. Mostly grown in perennial monocultures, pest/disease problems of apple are varied and complex in nature. Such situations have led to repeated and excessive use of chemical pesticides, which has resulted in the development of resistance in the pests and also to environmental pollution. Among 96 recorded diseases are apple powdery mildew, scab, blotch, cankers and root rots while 122 insect-pests include mites, scale, aphids, fruit fly, fruit borers and caterpillars; altogether, huge losses are incurred. Total annual losses are about 28-36% despite expenditure of approximately INRs 32000-40000/ha.

Adverse climatic conditions have emerged as major constraints for apple productivity, and have led to increased pest/disease populations in the last few years. Prolonged dry weather from the second week of April to the second week of August, has led to the frequent incidence of mites (100%). San Jose scale (15 - 26%) and woolly apple aphid (8 - 24%). Four species of mites were recorded infesting apples. The biology of the European red mite and the two-spotted mites have been studied in relation to plant phenology and weather conditions. The two-spotted mite developed faster (10 generations/year) than the European red mite (8 generations/year). Cumulative mite feeding for 750 - 1750 days resulted in a 2.4 - 40.2 % reduction in return bloom and a 5.4 - 50.4% reduction in fruit set in the following year. The natural population of predatory mites was also abundant and the ratio was around 60:40. The regression equations developed were used to forecast mite populations.

Biology of the San Jose scale revealed up to 6 generations in severely infested orchards. The promising predator of most apple insect pests, viz. coccinellidae, was monitored for its predatory activities to woolly apple aphids. Repeated sprays of horticultural mineral oil are effective at controlling the pest population as well as conserving predators within an IPM strategy.

In the integrated forecasting system for predicting apple diseases, a structural model on pathogen life cycles revealed leaf fall as the main scab disease criterion. The model validated that leaf fall occurring between 25 October and 15 November led to successful development of overwintering fungal stages within 55 - 68 days (i.e. from 20 December to 20 January) and that mycelium colonization and incubation is completed on the orchard floor. After colonization and incubation, rain/moisture initiates fertilization at around 4°C. Successful fertilization took 35 - 40 days at 8 - 10°C for the formation of pseudothecial initials. The asci and ascospore maturation took 132 - 140 days and spores were released between the first weeks of March to around 25 May. The study indicated highly synchronized behaviour of the host and the pathogen to the weather conditions and hence a mathematical model for annual forecast was developed.

A recent outbreak of apple blotch disease (caused by *Marssonina* and *Alternaria*) was concluded to be due to a combination of leaf ageing followed by rains that reduced the high temperature during the summer months to around 20°C followed by a period with relative humidity above 90%, which triggered the disease epidemic. Integrated and monitored sprays of fungicides have been devised for spring and summer diseases whereas an overwintering spray of 5% urea and Bordeaux mixture (1%) are a very effective IPM strategy to eradicate most of the foliar diseases on apples.

However, there is global concern about the overuse and misuse of chemical pesticides, which has resulted in adoption of Integrated Pest/Disease Management (IPM) for containing pest and disease problems. IPM is an eco-friendly approach encompassing cultural, mechanical, biological and need-based use of chemical pesticides. From a practical viewpoint, biological control may be considered as utilization of natural enemies (parasitoids, predators and pathogens) to reduce the damage inflicted by the pest to a tolerable level. These strategies, introduction, augmentation and conservation in one form or another have been exploited against several pests of temperate fruits, viz. the San Jose scale, mites, woolly apple aphid, white grubs, Indian gypsy moth and codling moth etc. A variety of potential natural enemies have been identified. The ectoparasitoid *Aphytis* sp. and the endoparasitoid *Encarsia perniciosi*, both identified as effective parasitoids, and *Chilocorus bijugus*, for the suppression of the San Jose scale, are the most promising. Likewise, an aphelinid endoparasitoid, *Aphelinus mali*, and various predators, coccinellids, syrphids and chrysopids play a vital role in suppression of the pest. Chrysopids, e.g. *Chrysoperla carnea* and *Chrysopa scelestis*, are also potential agents for aphid control. In orchards receiving low or no application of insecticides, the phytophagous mites can be checked by their predatory mites species *Amblyseius fallacis* and *Zitzellia mali*. Habitat management in horticultural crops through biological measures can be made more effective particularly by adopting conservation and promotion of natural enemies in the crop ecosystem. Indiscriminate use of broad spectrum pesticides (mainly insecticides) has led to the emergence of more complex problems such as the development of resistant pest species and the emergence of secondary pest outbreaks (like white scale and spider mite) in apple orchards.

It is evident that most horticultural crops have been subjected to indiscriminate use of chemical pesticides over the years, resulting in the killing of predators and bio-agents, and the break-down of the soil microbial system, which make nutrients available to the plant system. Adopting integrated pest management strategies can circumvent the problems posed by the use of pesticides. Only those pesticides that have a good pest management ratings, which are less toxic to non-target species and have lower persistence in the environment should be recommended and used. This approach is greatly warranted for an integrated crop production system in the agro-ecologically diverse Hill and Mountain Ecosystems.

Occurrence and management of root rot disease of *Panax notoginseng* in China

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Panax notoginseng is an important medicinal herb cultivated only in the limited mountainous areas of Yunnan, China. However, its production is severely limited by disease because of the length of propagation in warm, humid, and shaded environments. Root rot is an increasingly serious problem due to a lack of proper rotation in recent years. Disease incidence ranges from 10% to totally infected. In view of its serious importance, studies on the causal microorganisms, infection cycle and management were undertaken. This paper presents some of the results obtained.

Symptoms and pathogenic microorganisms

A survey indicated that the symptoms of the disease are expressed as dry chap rot, medullary tissue rot, slow yellow rot, stem base dry rot, rapid wilt rot and wet rot. Among these, slow yellow rot and rapid wilt wet rot were the most commonly occurring symptoms. Dry chap rot, medullary tissue rot and stem base dry rot are new records for symptoms of the disease. Isolation from diseased tissues indicated that *Cylindrocarpon destructans*, *C. didymum*, *Fusarium solani*, *Phytophthora cactorum*, *Phoma herbarum*, *Monilia* sp., *Verticillium* sp., *Trichoderma* sp. and *Rhizoctonia solani* were involved in the disease. *In vitro* pathogenicity tests demonstrated that *C. destructans*, *C. didymum*, *P. cactorum*, *P. herbarum* and *R. solani* could induce disease symptoms at varying levels. Field inoculation tests showed that *P. cactorum* and *P. herbarum* were the most pathogenic fungi, leading to disease incidences of 48.4% and 50.0%, respectively. It is considered that *C. destructans* and *C. didymum* are the two most important fungi causing *P. notoginseng* root rot diseases because of their wide distribution, high frequency of isolation, and close relationship with the dominant symptom.

Latent infection of seedlings

Investigation of infection sources is vital to effective management of the disease. It is known that the two most important fungi can survive in soil for long periods and soil disinfection with chemicals is effective in reducing incidence of the disease. However, this study considered whether infected roots on transplanted seedlings might serve as another important infection source. To confirm this hypothesis, symptomless seedlings were selected visually from among those used by farmers for transplantation at various planting locations. Dilution plating and tissue isolation methods were used to check for the presence of *Cylindrocarpon* species on the roots. Results indicated that *Cylindrocarpon* spp. could be isolated from symptomless root surfaces of all four samples taken from different plantations, with populations ranging from 4.0×10^4 to 2.3×10^5 CFU/root.

Cylindrocarpon spp. were not isolated from surface-disinfected tissues of the sprout and stem base but were from fiber, rhizome and tuber tissues. Isolation frequencies from tubers ranged from 1.1 to 16.8%, indicating that seedling quality at different plantations was variable. This study demonstrated that latent infection by root rot disease of notoginseng was quite common. In view of this, it is strongly recommended that seeds and seedling roots are chemically and/or biologically treated in order to prevent latent infections and to reduce the population of *Cylindrocarpon* spp. on seedling roots to reduce disease incidence.

Management of the disease

Integrated management of the disease was performed using soil fumigation, and seed and seedling treatments with chemical fungicides and biocontrol bacteria. Results indicated that soil fumigation with Dazomet at 30~40g/m², K-Vapam at 40~60g/m² and CaCN₂ at 30~90g/m² could reduce the populations of soilborne fungi, bacteria, nematodes and weeds by 90~99%. Among the three fumigants, Dazomet gave the highest mortality rate ranging from 96.7% to 99.9%. Field experiments showed that soil fumigation with Dazomet could increase the total number of first and second class seedlings by 29.5% and fresh weight of total seedlings by 53.4% in comparison with non-fumigation. Greenhouse experiments indicated that treatment of transplanting seedlings with a mixture of *Bacillus subtilis* G3 (an effective biocontrol agent of plant fungal diseases) and chemical fungicides, led to 90% control efficacy which is higher than given by treatment with either G3 or chemicals alone.

Experiments in a field where *P. notoginseng* had been continuously monocultivated for eight years, demonstrated that incidence of the disease could be kept to around 5% for one year old seedlings and 7.7% for two year old seedlings when seed/seedlings were treated with a mixture of G3 and Ronilan in combination with soil fumigation with Dazomet at 30g/m². Further tests on the effects of different mixtures of chemical fungicides indicated that Mix. 1 (Hymexazol : Bismethiazol : Validamycin in the ratio 1:1:1 (w/w)) and Mix. 2 (Carbendazim : Bismethiazol : Validamycin in the ratio 1:1:1 (w/w)) were very effective, giving rise to disease incidences of 6.8~7.8% for one old seedlings and 10.3~10.6% for two year old plants, which are much lower than in the controls. This research has revealed that soil fumigation plus seed/seedling treatment with biocontrol microorganisms and selective chemical fungicides could be used in combination for effective management of the root rot complex of *P. notoginseng*.