Session 3C

Advances in the Diagnosis and Forecasting of Plant Diseases

Chairman & Session Organiser:	Professor Andreas von Tiedemann *University of Göttingen, Germany*
Platform Papers:	3C-1 to 3C-5
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Developments in forecasting models for integrated disease control

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The present trend in crop protection against plant diseases is to limit the use of chemicals, by integrating host resistance with agricultural practices and non-chemical applications, and by spraying chemicals only when necessary. This approach implies an increase in the level of complexity and it also imposes on farmers the need for wider and deeper knowledge. There are two different tools for achieving this goal: decision support systems (DSSs) and warning services (WSs), which are both designed in such a way as to provide farmers with useful information at the right time, at personal and collective level, respectively. Disease models are a key prerequisite in developing both these tools.

The first disease models were developed following an empiric approach, with simple tools showing relationships between particular stages of the disease and the concomitant weather conditions. Developments in weather monitoring and automatic data processing had a relevant role in increasing the number of models and their complexity. Nevertheless, empiricism predominated for a long time. Relevant improvements were obtained with the mechanistic (explanatory) dynamic models, which are able to explain the relations within a pathosystem and to describe the way in which the system, and its internal relations, change over time as an effect of the influencing variables (weather conditions, host characteristics, control measures, etc.). Accuracy and robustness of these models were significantly increased compared to the empiric ones: models for apple scab infection periods and for oosporic infections with grape downy mildew are examples of these improvements. The models of this "new generation" make possible the prediction of future events of the disease based on information about the past or current state of the pathosystem. Therefore, forecasting key points of epidemics, like outbreak or increase in intensity, consists in identifying previous events and the relationship between past and future events.

Model building is a long, complex and expensive process which requires considerable funds, and collaboration between scientists (biologists, mathematicians, statisticians etc.). It is a step by step procedure with four main steps.:
i) Recognition and targeting of the specific phytosanitary problem (object-oriented modeling). It includes a flow of information from expert users (technicians, consultants, growers), an accurate analysis of the pathosystem related to the specified problem, and the organization of available knowledge in a relational diagram, according to the system analysis procedure. This is a key step as models may be aimed at forecasting specific aspects of epidemics, particularly those which are crucial for crop protection; if forecasts concern aspects of little significance in decision making, the model will be of little benefit.
ii) Design of the model and verification. Design includes the definition of both the conceptual and driving models, which are the mathematical cores representing the quantitative relationships relating parameters and variables acting in the model. Verification is the process of testing whether the behavior of each mathematical model represents the relationship under study reasonably well. This is a critical step as the model may incorporate all the necessary information for accurate prognosis; it concerns the host (growth stage, resistance to disease etc.), the environment (meteorological conditions, soil characteristics or cultural practices), and the pathogen (inoculum level, disease intensity).

Selection of information to be inserted is a crucial point. Reducing information could reduce accuracy, while increasing it could increase complexity, time and costs in both elaboration and use. The goal is to select the minimum amount of information needed to account for the greater part of pathosystem/disease variability.
iii) Validation and evaluation include the procedures for testing the agreement between model outputs and reality, and methods to check the computerized model for its practical use.
iv) Improvement and enlargement, resulting from new needs suggested by the model users.

DSSs are computerized tools designed to encapsulate experts' knowledge of agricultural situations; they contain decision guides that consist of procedures assessing specific risk factors at the plot level (like disease incidence in the crop) and actions to take based on models designing probable future scenarios starting from these assessments. In general, there is a poor acceptance of model-based DSSs in practice, for a variety of reasons: i) failure to support more than one or a few problems; ii) lack of computer expertise among the users; iii) system complexity; iii) use of inputs that the grower cannot easily provide; iv) difficulty in showing cost benefits.

WSs operate following an indirect warning. Information on crop protection is prepared for homogeneous geographic areas and given to the farmers by local newspapers, television or radio, telephone or other information technologies; farmers comply with them in an autonomous way. At the present time, this appears to be the best strategy to reconcile two different aims: i) contain the costs of WSs, and ii) serve the maximum number of farmers as possible. Warnings are usually prepared by a team of technicians, who analyze the available information (about weather, crops, pests, diseases, etc.) and, on the basis of their experience, elaborate suggestions for optimal crop protection. The use of forecasting models in preparing these warnings is not yet sufficiently widespread for several reasons. There is a lack of models for some prevalent diseases of widely cultivated plants, or the available models do not perform well under local conditions. Not much effort has been devoted to validating models elaborated in different areas, though some examples show that there is potential for profitable use of models elaborated elsewhere. Furthermore, all the models need meteorological data as their main input and many of them require additional information on the crop or pest/pathogen to perform at their best. The input data must be collected accurately at many representative locations, and they must be available in real time to run the models. This requires instruments, people, and a high level of organization, which are not available everywhere. Finally, many persons involved are not yet used to using models and are cautious in trusting model outputs. Frequently, this attitude is mistaken as the model outputs do not replace experts in decision making, but they support them by increasing the available information.

The following actions should be undertaken to solve these problems: i) devote further efforts to develop WSs and, where necessary, the services which support their use, either by increasing the financial support or by re-organizing the available resources; ii) promote and support research activities aimed at developing the knowledge necessary for preparing warnings, with emphasis on model elaboration and validation; iii) promote a 'warning culture' among the end-users; iv) stimulate the exchange of experiences between the WSs operating in neighboring districts, with the aim of giving homogeneous warnings; v) give chemical companies a joint interest in the warning-based approach; vi) improve the efficiency in spreading warnings by introducing new information technologies, especially the web.

A web-based information system for plant disease forecasting based on weather and soil data at high spatial resolution

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Introduction

Disease forecasts can assist crop growers in determining when or whether to spray fungicides. Recent advances in information technologies provide good opportunities to improve the quality of disease forecasts in terms of spatial and temporal resolution, and to allow growers maximum time for preparation and application of appropriate disease management techniques by delivering disease forecast information instantly after its generation. This paper describes, briefly, a web-based information system for plant disease forecasts that is currently under development in Korea. We aim to develop a forecasting system that can provide farm-specific forecast information on possible occurrence of various plant diseases on a real-time basis, so that farmers can have disease information for the crops on their own farms whenever necessary. Currently, we are in the process of enhancing the spatial resolution up to 240m by 240m. The information system generates hourly or daily warnings of major diseases of rice, apple, pear and pepper based on weather and soil texture data.

System structure

The information system, which is implemented on a PC server with the Linux OS, consists of four components including a weather data acquisition system (WDAS), data storage system (DSS), job process system (JPS), and web service system (WSS). WDAS collects hourly weather data from two sources: the Korea Meteorological Administration (KMA) and automated weather stations (AWS) installed in paddy rice fields, apple and pear orchards, and pepper fields at 62 locations in South Korea. The methods of data transmission from the sources to WDAS vary depending on the sources of weather data. Data files from the KMA weather service, which contain synoptic and AWS weather data, and 6 hour-, 3 day-, and weekly-forecast data, are transferred by the file transfer protocol through the Internet. In the case of hourly data measured by the 62 AWSs installed on farms data are transmitted through either telephone modems or CDMA modems. All weather data retrieved by WDAS are processed by respective processors depending on data types, and stored separately in four different databases of the DSS (canopy weather DB, KMA synoptic weather DB, KMA AWS weather DB, and KMA weather forecast DB). In addition to weather data in a MySQL database, the DSS contains mesh weather data and disease forecast data in map image files, disease diagnosis and agrochemical information in a MySQL database, and soil texture data in map image files. The DSS contains all data generated by WDAS and JPS. Using weather data in the MySQL database, JPS processes scheduled jobs such as weather interpolation and disease forecasting. Weather data from the DSS are interpolated to produce mesh weather date at a spatial resolution of 240m by 240m. The mesh weather data are in turn used by disease forecasting models to generate

disease forecasts at the same spatial resolution. Both mesh weather data and disease forecast data are transformed into map images, which are then stored in the DSS. The job processing is executed hourly or daily by the *cron daemon* in the Linux OS. In JPS, disease forecasting models operate as independent modules so that we can add or modify forecasting models without much difficulty. Currently, forecasting models that are available for the system include blast, sheath blight and grain rot of rice, scab and white rot of apple, scab and rust of pear, and anthracnose and late blight of pepper. All forecasting models generate risk levels of infection based on hourly and daily weather data. In the case of pepper late blight, soil texture data are used in addition to daily weather data. WSS retrieves the weather and disease forecasting data stored in the DSS upon request by users through the web browser. All map images of weather and disease forecast data can be displayed quickly on the web browser because the WSS retrieves the map images already stored in the DSS instead of rendering them on a real-time basis. The WSS was created with PHP and JavaScript.

Conclusions

The disease forecasting systems for rice, apple and pear are currently available for farmers at http://nongupepi.gyeonggi.go.kr, www.apple.go.kr, and www.pear.go.kr, respectively. In the case of the rice system, which was developed in 2006, infection risk information on blast, sheath blight, and bacterial grain rot is provided at a spatial resolution of 240m by 240m. The rice system has been available to the public since January 2007. The apple and pear systems have been available to the public since 2004 and 2005, respectively. In both of these systems, the spatial resolution of disease and weather information is not as fine as in the rice system but representative information on diseases and insects, including forecasts and field monitoring data, are available for individual local districts. More than 5,200 and 1,300 users have registered for the apple and pear systems, respectively, and the registered users can obtain more user-specific information than the general public. The disease forecasting system for pepper is almost ready for public service and will provide forecasts on anthracnose, late blight and tobacco bud worm at the same spatial resolution of 240m by 240m.

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Detection of pathogens in plants: new technologies for old challenges

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Timely and reliable diagnosis is a crucial prerequisite for decision-making in the integrated control of plant disease. Growers need fast and robust assays for economically relevant pathogens threatening their crops but they can afford to spend only a fraction of the costs incurred by diagnostic assays in human and veterinary medicine. Recent technical advances achieved in bioanalytics may provide solutions to this daunting task.

The aim of diagnostics is to establish whether a particular pathogen or a group of pathogens is present in the sample and, in the case of quantitative assays, to determine the titer or biomass of the causal organism or virus. Because speed is a central requirement, physiological and cultural techniques are unsuitable for routine large-scale tests. Enzyme-Linked Immunoassay (ELISA) became the method of choice in the diagnosis of plant diseases soon after its invention in the 1970s and remained unrivalled for nearly twenty years, before the introduction of the Polymerase Chain Reaction (PCR) started to erode its monopoly.

Both ELISA and PCR draw their specificity from the information content of biological macromolecules, and they are therefore grouped with molecular diagnostics methods. ELISA is based on the interactions of antibodies with antigens, usually derived from surface structures of the pathogen. The specificity range of an antibody cannot be systematically controlled; in practice the antibody with the best response is selected empirically. Monoclonal antibodies can be used to limit undesirable cross-reactions but their sensitivity is usually inferior as compared to polyclonal antibodies. In theory, this drawback can be overcome by combining a set of carefully selected monoclonal antibodies but this is too costly to be used for diagnostic purposes. ELISA is principally unsuitable for the detection of pathogens which consist entirely of nucleic acid (viroids). PCR is based on the amplification of a DNA sequence (generated from RNA by reverse transcription in the case of RNA viruses) by a pair of oligonucleotides (primers) binding to specific target sites flanking the amplified fragment. Because areas used as a binding site for the primers can be selected based on the required specificity range of the assay (e.g. formae speciales, a species, a group of species), the specificity of PCR can be controlled. For example, PCR based on the amplification of the Tri5 gene, which encodes a key enzyme of trichothecene biosynthesis, is used in diagnostic tests for several *Fusarium* species producing mycotoxins of the trichothecene family. Because principally a single molecule can be amplified, PCR offers the ultimate sensitivity, surpassing ELISA by several orders of magnitude. One of the modern PCR variants, called digital PCR, can be used to literally count target molecules.

A shift from ELISA to PCR has occurred in medical diagnostics and to a large extent also in veterinary medicine in the last decade. In plant disease diagnostics, this process has been slowed down by a cost constraint. ELISA will probably survive on the market for plant disease diagnostics during the next five or ten years because it is cheap, easy to perform and well established in plant protection groups. There is no doubt, however, that nucleic acid based methods will eventually supersede ELISA because they possess genuine potential for

further advancement, while the development of ELISA is essentially completed. Miniaturization, automation and the use of alternative detection principles make ELISA faster and more sensitive, but immunochemical techniques will never achieve the sensitivity and specificity of PCR-based nucleic acid detection.

Companies selling diagnostic ELISA kits for the detection of plant pathogens are already examining their options to manufacture PCR kits. The profit margin achievable with kits for classical PCR is low, because once the sequences of species-specific primers enter the public domain, everyone can buy these primers at low costs and make up their own assays using standard laboratory equipment. Apart from chemicals and disposables for DNA extraction optimized for plant matrices, the specific value contributed by a company producing PCR kits is restricted to DNA standards, limiting their potential for generating revenues. Future markets for diagnostics are likely to shift from manufacturing kits and equipments to providing full laboratory services. The introduction of real-time PCR to routine diagnostics and the development of new methods such as miniaturized 'on chip' PCR with electrochemical detection, which require significant investments into instrumentation and advanced know-how for implementation and troubleshooting, will reinforce this process. A growing market for the detection of genetically modified material (GMO) in plant products requires a real-time PCR approach and is likely to promote the proliferation of diagnostics services, too, because the same technology is used for both purposes. To appreciate the relative efforts devoted to the development and application of major technologies in agriculture diagnostics, it is instructive to compare the number of research articles listed in the AGRICOLA database (Table 1).

Table 1: Journal articles in AGRICOLA referring to one of the major molecular diagnostics techniques in their titles

Publication year	ELISA	PCR	real-time PCR
1980	60	-	-
1990	60	7	-
1995	55	230	-
2000	66	288	2
2005	75	428	126

Techniques based on the detection of nucleic acids different from PCR have been used in the past and are still being used in research but they are not competitive with PCR commercially. The last company that offered diagnostic services for plant diseases based on DNA hybridization ceased operating a couple of years ago.

Which innovations may revolutionize plant disease diagnostics in the future? Nondestructive analysis and remote sensing of pathogens would advance agricultural diagnostics to a qualitatively new level, facilitating on-demand application of chemical protection as required by the concept of precision agriculture. As difficult as this task may appear, it is, in principle, not out of reach. The exploitation of optical methods (reflectance and fluorescence) to this end has not been successful so far but image processing technologies in combination with artificial intelligence offer new options. On the other hand, on-site detection of specific volatiles with diagnostic value by mass spectrometry, inspired by anti-terrorism tools and military research, might open new avenues for agricultural diagnostics and bring the idea of precision agriculture closer to reality.

Taking molecular diagnostics into the field – the PortCheck experience

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Abstract
In the diagnostic laboratory, there has been an ongoing need to develop ever more rapid and sensitive diagnostic methods, generating the impetus to develop technologies that are both generic and able to complement traditional skills and techniques. A further driver is the need to make more rapid decisions at the point of entry of imported material and anywhere where control measures may need to be implemented, thus point of inspection or field-testing is also becoming more important. Whilst antibody-based lateral flow devices are still the mainstay of field testing, real-time PCR is starting to make inroads as portable instrumentation becomes more readily available. Progress in the field detection of plant pathogens and pests is reviewed, and results presented from the EU FP6 project PortCheck – 'Development of generic on-site molecular diagnostics for EU quarantine pests and pathogens'.

Introduction

The principal objective of the project was to support the Council Directive 2000/29/EC on protective measures against the introduction into the Community of organisms harmful to plants or plant products, and against their spread within the Community, by enhancing the ability of Member States to detect plant quarantine organisms at the point of inspection. While the ideal is to be able to detect all problems at the site of inspection, most current methods can only be performed in laboratories.

Current methods of testing for quarantine pathogen/pest detection or identification involve the use of serological methods (using antibodies), morphological methods (sometimes involving culturing), physical/chemical methods or certain molecular methods (using nucleic acids). Molecular methods offer, perhaps uniquely, a generic technology to detect a wide range of pests and diseases confronting the inspection services. All organisms share the possession of nucleic acid as their common underlying feature, thus the same format of test could be used for any pathogen or pest. Methods using the polymerase chain reaction (PCR) in particular are extremely sensitive and can be used to detect very low levels of pathogen, and real-time PCR removes the need for post PCR manipulations. The need for rapid and robust detection of bio-warfare and bio-terrorist agents on the battlefield has led companies, such as Cepheid, to pioneer the development of portable and robust real-time PCR machinery. The equipment is effectively a portable thermocycler and fluorescence detection system that can be run off a car battery, and is capable of generating a result within 20-30 minutes.

Results and discussion

A number of new assays were developed and these along with existing assays were validated for use on the SmartCycler platform for a range of pathogens or pests as follows: *Phytophthora ramorum, Bursaphelenchus xylophilus, Ralstonia solanacearum, Clavibacter michiganensis ssp. sepedonicus, Tilletia indica, Potato spindle tuber viroid, Thrips palmi, Synchytrium endobioticum, Meloidogyne chitwoodi/fallax, Guignardia citricarpa and Globodera pallida/rostochiensis.*

The other key components for effective field testing are sample disruption, nucleic acid extraction and the use of ambient stable reagents, each of these were also resolved in the course of the project. A portable battery-powered sample disruption system was developed which allowed the homogenization of samples in disposable plastic bags. A nucleic acid extraction method was developed based on novel ChargeSwitch (Invitrogen) technology, that enabled extraction of eight samples in less than 10 minutes without the use of any laboratory equipment. Finally stabilized reagents based on Cycleave chemistry were developed for *Phytophthora ramorum* that could be stored at ambient conditions and contained all the reagents needed to perform the real-time PCR test (Takara Bio).

Following completion of the development work, staff from 21 different laboratories around Europe were trained to complete the method for the detection of *Phytophthora ramorum.* A method performance study was then completed by supplying each of the laboratories with equipment, reagents and a panel of standard samples for testing for *Phytophthora ramorum.*

The results show that the method could be performed effectively with very minimal training, only three sets of data were found to be outliers, however, in each case the controls also failed and the results were reported as such. Initial analysis of the results show that the method performed as well as standard laboratory-based real-time PCR performed by highly trained diagnostic staff.

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Detection and quantification of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida*

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The potato cyst nematodes (PCN) *Globodera rostochiensis* and *G. pallida* occur in many potato growing regions world-wide, including the UK, where they cause damage valued at over £50m per annum. Over 60% of potato fields in the UK are infected with *G. pallida*. Controlling PCN is problematic as a consequence of the withdrawal of nematicides, the lack of resistant cultivars and the increasing prevalence of *G. pallida*.

Sampling, detection and quantification of PCN in soil remains of fundamental importance in ensuring appropriate management strategies. To improve the efficiency of detection, a quantitative PCR assay has been developed. The sensitivity of this assay in detecting PCN in soil has been assessed and the assay can be used to quantify the presence of either or both species. We are also examining the efficiency of detecting PCN in 'floats' that have been obtained from soil samples. The assay has also been applied to an experiment to determine how the two species compete on potato lines with differing degrees of resistance. This quantitative assay for PCN has the potential to provide assessments of species composition and quantities which will be valuable in enhancing the efficacy of decision support tools.

The assay is based on targeting the ribosomal internal transcribed spacer (ITS) repeats, which have high homology within each species. Primers were designed for each species that amplify a polymorphic region to which specific probes were constructed. DNA from each species was obtained and a dilution series generated. After optimization, the primers and primer/probe sets were tested with the DNAs separately or in mixtures. The results from assessments of template DNA, obtained from cysts from either species, in a 10-fold dilution series showed that the primer probe combinations were specific for the two species. The assay showed clear positive detection of *G. rostochiensis* or *G. pallida* DNA on their own or when mixed with DNA of the non-target species. Over the range 1ng to 0.001ng there was a clear linear response. However at the two lowest concentrations (less that 0.001 ng), detection was minimal or there was no detection.

The assay was then used to determine its utility in estimating the relative composition of species mixtures by first making artificial mixtures of cysts. A good correlation was obtained between the estimated ratios of the two species and the ratios in the original mixtures. The technique was also used to assess the composition of species mixtures in an experiment designed to assess intraspecific competition between the two species on susceptible or partially resistant hosts.

Next, the assay was used to determine if PCN could be detected in soil samples. DNA was extracted from 60g of soil, using the method of Van de Graaf *et al.* (2003), to which known numbers of cysts of *G. rostochiensis* or *G. pallida* had been added. The assessment of PCN where the template DNA was obtained directly from soil has shown that from a 60g soil sample artificially inoculated with five or more cysts, detection was reliably obtained and specificity maintained. The ability to detect 1 cyst was inconsistent.

Normally to assess cyst numbers, the cysts are extracted from soil by flotation and are recovered along with some of the other organic matter present in the soil. This sample or 'float' then needs to be visually inspected which is often time consuming. Extracting DNA from the float is being investigated and initial results indicate that one cyst can readily be detected. This part of the study is being conducted with SASA who are supplying floats typical of those obtained during the statutory potato seed certification scheme and where detection of low numbers of eggs is imperative.

The experiments conducted so far indicate that this technique is able to detect and quantify both species of PCN. The method has proved useful in studies of PCN species mixtures, which hitherto have been limited by the difficulty of distinguishing the species visually due to the high degree of morphological similarity. However, more work is required to establish the lowest possible level of detection, particularly directly from soil samples which, at the moment, do not meet the stringent requirements of statutory testing of land for the production of seed potatoes. Assessment requiring this level of detection may well be better met by extracting DNA from the 'float' obtained from soil samples. Whilst the sequences of the primers and probes used are specific, and have been checked *in silico* against other cyst nematode sequences, there remains the need to experimentally verify the specificity to ensure that other cyst nematode species are not detected by these primer/probe sets.

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A forecasting model for the initial incidence of citrus scab disease (*Elsinoe fawcettii*) on spring flushes

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Citrus scab diseases cause severe deformation of foliage, stunting of certain citrus rootstocks, and fruit blemishes that reduce the value of the fruit for the fresh market, and are important wherever susceptible cultivars are grown for fresh fruit in humid areas. Citrus scab pathogens have been assigned to two species, *Elsinoe fawcettii* and *E. australis*, and pathotypes of each species have been described (Tan *et al.*, 1996; Hyun *et al.*, 2001).

Citrus scab pathogens survive periods without susceptible tissue in pustules on old leaves and then infect new flushes in the next season (Timmer, 2000). Citrus foliage is susceptible to scab for only a short time after emerging from the bud and becomes resistant at more than 25% leaf expansion. Control of disease on the spring flushes, and its timing, are important because it is inoculum from lesions on the spring flushes that infect the fruit.

Table 1. Carryover of conidia produced from lesions on leaves from the previous year in relation to temperature and duration of humidity periods

Temp.	No. of conidia per lesion Duration of humidity period					
	0 day	1 day	2 days	3 days	4 days	5day
10 ℃	0.0±0.0	0.0±0.0	0.0±0.0	1.4±0.8	8.9±8.1	40.9±46.9
15 ℃	0.0±0.0	0.2±0.3	8.3±6.3	27.1±15.2	461.9±187.5	> 1500
20 ℃	0.0±0.0	1.6±2.2	130.6±130.6	814.1±523.1	418.5±406.7	> 1500
25 ℃	0.0±0.0	6.3±7.1	113.5±96.5	694.4±687.5	> 1500	> 1500

First symptoms on one-fourth expanded leaves appeared 36 h after inoculation at 25°C, and after three days at 20 and 30°C. On the other hand, symptoms first appeared six days after inoculation at 15ºC but no symptoms appeared at 35°C. A humid period of three hours after inoculation was sufficient for disease induction (Table 1).

These results are consistent with what has been seen in the field in recent seasons, with respect to the observed conditions for inoculum production and initial disease induction on the spring flush. Spores were produced and infected the young shoots in open fields on about 4 May in 2004 and 1 May in 2005, and first symptoms appeared on 10 May and 5 May, respectively. Based on these data, a forecasting model was derived as $f(x) = 1\text{-}\exp[-(x/\beta)^{\delta}]$, where β : 1.0511, δ : 8.0136. This model explained the initial incidence of scab disease on spring flushes of citrus in Jeju Island in 2005 to 2007.

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PearScab: software for forecasting pear scab development on a real-time basis

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Introduction

Pear scab, caused by *Venturia nashicola,* is one of the most serious diseases of pear in Korea. As the major control strategy, fungicides are usually applied to pear crops on a regular basis during the growing season. Disease forecasting is important to enhance the effectiveness of fungicides and to minimise the numbers of sprays applied. This paper describes PearScab, a software system to determine infection risks of pear scab based on hourly temperature and wetness period.

Infection risk model

Because *V. nashicola* has epidemiological characteristics similar to *V. inaequalis*, the pathogen of apple scab, we applied the Mills model (Eq. 1) to determine the minimum wetness periods (W) required for pear scab infection under certain temperature (T) (MacHardy et al., 1989).

$$W = 86.3749 - 18.0034T + 1.5703T^2 - 0.062T^3 + 0.0009198T^4$$

(Eq. 1)

The hourly infection risk is calculated by taking the reciprocal of temperature-dependent minimum wetness period determined by the Mills model. It is zero when the plant surface is dry. Thus, the hourly infection risk (IR) at time t is calculated as follows:

$$IR_t = \begin{cases} \dfrac{1}{W_t}, & \text{if wetness duration} > 0 \text{ at time } t \\ 0, & \text{if otherwise.} \end{cases}$$

The hourly infection risk is accumulated every hour if hourly rainfall was less than 4 mm and if the wetness period continued without interruption by a dry period of longer than or equal to 12 hours. Once such unfavorable conditions occurred, the accumulated infection risk is reset to zero. Thus, the accumulated infection risk ($ACCIR$) at time t is calculated as follows:

$$ACCIR_t = \begin{cases} ACCIR_{t-1} + IR_t, & \text{if rainfall} < 4 \text{ and accumulated dry hour} \le 12 \text{ at time } t \\ 0, & \text{if otherwise.} \end{cases}$$

PearScab software

Based on the logic of the infection model, a computer program PearScab running on a PC was developed using Microsoft Visual Basic 6.0. It calculates hourly *IR*s and hourly *ACCIR*s using hourly weather data. Acquisition of weather data to PearScab can be done in two ways:

1) downloading hourly weather data via the Internet connection from a centralized weather data management server collecting weather data every hour via telephone or wireless CDMA modem from automated weather stations (AWSs) installed in pear orchards;

2) collecting hourly weather data directly from stand alone AWSs installed at individual orchards. Currently, PearScab is programmed to run with AWSs with a CR10X datalogger (Campbell Scientific, Inc., USA). PearScab provides hourly and daily weather data, and hourly *IR*s and *ACCIR*s, of multiple sites in table and graph format (Table 1). If *ACCIR* is higher than a critical level of infection risk, PearScab gives a warning of pear scab infection. The weather data acquisition and disease forecast are performed automatically on a real-time basis. The critical level of infection risk can be set by users depending on their own attitude to infection risk.

Table 1. Information provided by the PearScab

Category	Time interval	Element
Weather monitoring information	Hourly and daily	Air temperature(°C), relative humidity (%), ground temperature (°C), rainfall (mm), leaf wetness duration (hr/hr), wind speed (m/s), wind direction (degree), solar radiation (MJ/m^2)
	Daily	Maximum, minimum, and average of air temperature (°C), relative humidity(%), and ground temperature (°C)
Disease forecasting information	Hourly	Infection risk, accumulated infection risk, base wetness duration hours (hr), infection warning message

Conclusion

PearScab software can be used by farmers for scheduling fungicide sprays. After a spray applied in response to an infection warning, farmers can omit sprays during the period that fungicide residues on the plants are still effective in the field even though subsequent warnings occur during that period. Analysis of historical data from 2002 to 2003 suggested that, by using PearScab warnings, it would have been possible to reduce fungicide sprays by four and five applications respectively.

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Use of data from a disease management service to develop protocols for disease forecasting and management in mushroom production

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Introduction

Worldwide, mushrooms are usually grown in specially constructed rooms. Despite this, the industry still has pest and disease problems, some of which gain entry to the system at the weak links, such as when the rooms are filled or the casing layer applied. Hence, events outside the rooms still have an impact on the efficient cultivation of mushrooms.

However, as cultivation does occur indoors, mushroom growers do have better options for physical exclusion, environmental management and containing problems than most other agricultural industries. Access to pesticides by the mushroom industry on an international basis is being restricted by a combination of pest/disease resistance and withdrawal of registration by legislative means. This reduction places more emphasis on physical exclusion and containment of problems, increasing the importance of understanding disease epidemiology.

Methods

In New South Wales, Australia, there are fifteen years of records of pest and disease incidence in the industry, although the data is often just presence/absence, with limited information on severity. We report here our first efforts to use a subset of these records to develop protocols to forecast the occurrence of a fungal disease of mushrooms, and to manage these occurrences. The disease selected was dry bubble, caused by *Verticillium fungicola*, mainly because we have been working on aspects of this problem for several years (Clift and Shamshad 2004). The software used to manage the data and to develop a working model was Netica Ver3, which is based on a Bayesian Belief Network (BBN). The software can be purchased from www.norsys.com .

The model

Briefly, a BBN is a graphical representation of how it is believed the system being modeled functions in terms of the main factors and how they interact. Real data is then fed into the model and the software then indicates how well your data supports the model. The model can also be validated against an independent set of data.

The data used to derive the model was from a detailed survey of pests and diseases in the Australian mushroom industry. There were data from four farms, two of which had problems with Dry Bubble, whereas the other two had virtually no incidence of the disease. At the end of the survey, each farm was rated for disease management skills, and this estimate was one of the factors, termed nodes in a BBN, used in the model.

Previous work (Clift and Shamshad 2004) indicated both flies and dust have roles in Dry Bubble epidemiology, so both these were included. Other factors were: fungicide and insecticide use, efficiency of physical exclusion and an estimate of the proportion of growing rooms infected with the disease.

The nodes and their states are:
Dust: True or False; *Flies*: nil, low, high; *Other rooms:* nil, one, two, three, four; *Disease management:* Poor, Indifferent, Good; *Physical exclusion:* Poor, Indifferent, Good *Insecticide use:* nil, triflumuron, diazinon; *Fungicide use:* nil, benzimidazole, Prochloraz; *Pesticide efficiency:* Poor, Indifferent, Good; *Initial Infestation:* True, False; *Outbreak in room:* True, False.

Results and discussion
Disease management and physical exclusion data were obtained from the survey. Pesticide efficiency was determined by the effectiveness of each pesticide treatment, including resistance issues. It was found that if this was not done, no more than 25% of the observed variation could be accounted for in the model.

Dust, flies, physical_exclusion and other_rooms were all considered to directly influence initial_infestation. Disease_management, insecticide_use, fungicide_use, flies and, pesticide_efficiency then determined if an initial infection became a disease outbreak.

Once the survey data had been read into the model, the Sensitivity Test module was then used to determine the relative importance of each of the factors and how much of the observed variation was accounted for. The largest single factor was *Other_rooms*, which accounted for over 20% of the variation. *Flies* and *Dust* accounted for a further 5-7% each and *Pesticide_efficiency* accounted for about 4%. However, if this latter factor is left out, the overall model is much less definitive.

To validate the model, it was used to forecast disease incidence for an independent set of disease incidence data.

Acknowledgements
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Forecasting of meadow moth (*Loxostege sticticalis* L., Pyralidae, Lepidoptera) in China

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Introduction

Meadow moth, *Loxostege sticticalis* L., is widely distributed in the northern part of the Northern Hemisphere, including North America, Europe and Asia. In China, this species predominantly occurs at locations farther north than 37 degrees northern latitude, and its population has shown a long periodicity of intermittent abundance. There were several outbreak years in the 1950s, followed by an intermission for more than 20 years. Then, another abundant period occurred during the later 1970s and early 1980s, and another intermission after 1985. Since 1989, the population has been growing, with recent outbreaks between 1999 and 2004, and with large numbers of spring adults and damaging populations occurring until this year.

Biological features, including long distance migration and diapause, make meadow moth an insect pest with variable abundance and its forecast is a prime objective of pest control. This paper summarizes research and application of meadow moth forecasting in China by the progress of investigating the migration paths of meadow moth populations, as the guiding factor for migratory insect forecasting.

Middle term forecast

The mid-term forecast is generally released 10-30 days before larval population formation. Adult observations are the major way of investigating the population state for the mid-term forecast. There are two common survey methods applied for meadow moth adults, which are black light catches and the collecting of disturbed moths during 100 steps walking in the fields. Black light has been applied in the meadow moth forecast for more than 30 years. The equipment consists of a 20 W black light tube and some supplementary fittings. The quantity and date of adults caught in black light traps and the disturbed moths collected during 100 steps walking in the fields are used as the main cardinal numbers in population analyses for forecasting the occurrence and size of the population and the period of damage.

Migration makes adult surveys complicated, and the development of female adults is also used as an important forecast index. Female adults from field catches are dissected and the development state of internal reproductive organs is observed and scored as four degrees, that is, the initial stage during pro-oviposition as the first, the later stage during pro-oviposition as the second, the initial and peak oviposition stages as the third, and the later oviposition and the end oviposition stages as the fourth degree (Sun *et al.*, 1991). It is considered in the population analyses that the adults of the first degree possessed powerful flight ability and mostly emigrated, the second degree possessed the possibility of migration or colonizing, and the second and third degrees formed the major reproductive potential of the population in the location of moth catches.

Meadow moth was the first insect observed by radar in China. Radar observations of spring adults were made in northeastern China, and used in the research and application of

meadow moth forecasts in 1999, in which the quantity and development of the airborne population were used as the basis of the forecast in the immigration area (Sun & Gao, 2000). Radar has not, so far, been a common tool in forecasting systems in China, but its potential has been confirmed, and experiments and research thereupon are in progress.

Long term forecast

Long term forecasts are released over 30 days before larval population formation. The larval stage in soil during the meadow moth life cycle is regarded as the main source for the next generation, while migration and diapause are also regarded as important factors in determining plague population occurrence. The investigation of meadow moth stages in soil depends on manual work. The quantity and distribution of overwintering populations in the field are used as the main basis of spring generation forecasts, and the field surveys of overwintering population are carried out in autumn and spring.

Short term forecast

The short term forecast gives 10 days warning of the formation of a potentially damaging population. The field surveys of eggs and lower stadium larvae are used in the population analysis, which are mainly based on observations and catches in the field.

Prospects for meadow moth forecast

Meadow moth is one of the target insects in China's pest forecasting systems. The common practice of forecasting requires complex inputs, which include investigations on the density and range of the target insect population, the collection of ecological data, the analysis and transfer of data and information. The forecasting system has been progressing with the development of connective science and technology, and the analysis of data and dissemination of information has been made suitable for present-day needs. The accurate assessment of natural insect populations is still critical, and some new techniques, like radar, satellite etc. will be applied soon.

Acknowledgements

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The canopy hyper-spectrum character of maize damaged by *Curvularia lunata* (Wakker) Boed, and yield loss estimation

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Maize leaf blight caused by *Curvularia lunata* (Wakker) Boed is a new epidemic disease in the maize growing areas of Hebei, Henan, Shandong, Liaoning and Jilin Provinces in China in recent years. It can cause huge losses in quality and yield of maize because most of the maize cultivars are susceptible to the disease once the temperature and the ambient humidity are above average in a particular year. It is very important to monitor the maize growth status and the occurrence of the disease. Remote sensing techniques are applied for various purposes, such as the monitoring and protection of agricultural fields, the estimation of yield in large-area crops, the monitoring of crop growth, the monitoring of agricultural and meteorological disasters, applying crop simulation models etc.

In this paper, the canopy hyper-spectrum character of maize damaged by *Curvularia lunata* (Wakker) Boed and its effects on yield were tested after artificial infestation with the pathogen on the cultivar Shenshi 3005 in the field in 2005 and 2006. The inoculation levels were 4, 8, 14, 30 pathogenic fungal spores per microscope field (40x ocular). The ASD Field Spec instrument, with a spectral region between 350nm and 1050nm, was used to collect the canopy reflectance spectrum at different damage levels and at different growth stages of maize. In addition, the disease index and the chlorophyll content were surveyed. The yield loss was measured at harvest.

The disease indices (DI) on maize leaves differed with the inoculation levels and the maize growth stages. The disease indices were 1.50, 4.33, 8.67 and 9.83 from the lowest to the highest inoculation levels in the tasselling stage (three days after inoculation with *Curvularia lunata*) and increased to 9.33 (uninoculated), 44.33, 74.33, 84.00 and 92.67 by the waxy ripe stage (36 days after inoculation), respectively.

The reflectance of canopy spectrum was decreased when the maize leaves were inoculated with *Curvularia lunata*. There was a significant negative correlation between the reflectance of canopy spectrum in the near-infrared (745-900 nm) region and the disease index. In order to remove the influence of the background interference, the data of the reflectance of canopy spectrum were changed to the first derivative. The maximum of the first derivative of the spectral reflectance also had a negative correlation with the disease index. The reflectance of canopy spectrum and its first derivative maximum were 0.655 and 2.3×10^{-2} when the disease index was 4.33 in the tasselling stage (three days after inoculation), 0.50 and 1.33×10^{-2} when the disease index was 28.3 in the pollination stage (13 days after inoculation), 0.31 and 1.10×10^{-2} when the disease index was 57.0 in the milk stage (23 days after inoculation), 0.21 and 0.57×10^{-2} when the disease index was 74.33 in the waxy ripe stage (36 days after inoculation).

The following linear regressions between the disease index (DI) and the reflectance of canopy spectrum were obtained: DI = -69.732 $\ln(r_1)$ - 25.462, r = -0.9817**, where r_1 is the spectral reflectance at the tasselling stage; DI = -90.589 $\ln(r_2)$ - 38.459, r = -0.9893** where r_2 is the spectral reflectance at the pollination stage; DI = -114.19 $\ln(r_3)$ - 93.625, r = -0.9220*, where r_3 is the spectral reflectance at the milk stage; DI = -69.65 $\ln(r_4)$ - 48.51, r = -0.9120*, where r_4 is the spectral reflectance at the waxy ripe stage. Correlaztions of Di with the first derivative maximum were: DI = -55.702 $\ln(p_{max1})$ - 204.79, r = -0.9588**, where p_{max1} is the first derivative maximum at the tasselling stage; DI = -57.97 $\ln(p_{max2})$ - 220.91, r = -0.9731**, where p_{max2} is the first derivative maximum at the pollination stage; DI = -75.207 $\ln(p_{max3})$ - 297.74, r = -0.9273*, where p_{max3} is the first derivative maximum at the milk stage; DI = -39.538 $\ln(p_{max4})$ - 148.41, r = -0.8819*, where p_{max4} is the first derivative maximum at the waxy ripe stage.

Maize leaf blight can cause reductions in chlorophyll content of maize leaves. The higher the disease index, the less chlorophyll was recorded. Compared to the control, the chlorophyll content was decreased by 0.11 mg/g, 0.16 mg/g, 0.19 mg/g, 0.31 mg/g at the early stage of maize leaf blight inoculation and decreased by 0.43 mg/g, 0.86 mg/g, 1.00 mg/g, 2.16 mg/g at the four inoculation levels at the terminal stage.

The disease caused a significant reduction in dry grain weight per plant. Dry grain weight per plant was reduced to 126.7g, 93.0g, 86.0g, 75.0g depending on the disease index. The yield loss ranged from 13.3 % to 48.7 % depending on the disease index. There was a significant negative correlation between the yield loss (L) and the first derivative maximum (p'(max)) of the reflectance of maize canopy spectrum at the waxy ripe stage. The equation was L = 53.8-3231.5p'(max), r = -0.84**.

This research indicates that a significant correlation exists between the canopy hyper-spectrum character of maize, the disease index, the chlorophyll content and the yield. The canopy hyper-spectrum character could be used in diagnosis of the disease status of the crop and to estimate the yield loss.

Molecular identification of the most common fungi associated with grapevine decline in Castilla y León (Spain)

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Introduction

Grapevine decline (eutypa dieback, esca, Petri disease, black foot, black dead arm, diplodia cane dieback) is responsible for important economic losses to the wine industry worldwide. These diseases, which affect vine wood, are associated with different species of fungi. Their external symptoms can be erratic - even asymptomatic infections are known - but in all cases the productive life of affected vines is shortened. Eutypa dieback, which is mainly associated with *Eutypa lata*, is characterised by slow-growing branches, shortened internodes, and sparse and chlorotic leaves. Esca, which is associated with *Fomitiporia mediterranea, Stereum hirsutum, Phaeomoniella* (*Pa.*) *chlamydospora* and *Phaeoacremonium* (*Pm.*) *aleophilum*, is identified by inter-vein chlorosis, leaf necrosis and wilting. Petri disease mainly affects young vines, causing stunted growth and dieback; *Pa. chlamydospora* and *Pm. aleophilum* are the main fungi associated with this problem. Black foot is associated with *Cylindrocarpon* spp. and is another disease of young vines; affected plants grow slowly, show reduced vigour, retarded sprouting, shortened internodes, and sparse and chlorotic foliage. Diplodia cane dieback and black dead arm are associated with *Botryosphaeria*-like species.

In Castilla y León, the *Botryosphaeria*-like species associated with vine decay are *Diplodia seriata, Diplodia mutila, Botryosphaeria dothidea, Neofusicoccum parvum, Dothiorella sarmentorum, D. iberica* and *D. viticola. Phaeomoniella chlamydospora, Phaeoacremonium aleophilum* and *Cylindrocarpon* spp. are also associated with vine decay in this area. It is believed that grapevines are already infected when planted, and/or that infection is spread via cuttings. No efficient method for screening grapevines in nurseries exists. The aim of the present work was to develop a simple, rapid, sensitive and specific method to detect the main fungi species associated with grapevine decline in Castilla y León.

Grapevine decline surveys were started in 2003, and samples were collected and analysed. Fungi were identified based on their morphological, cultural and sporulation characteristics; identifications were confirmed by sequencing a region of the internal transcribed spacers (ITS) of the ribosomal DNA genes since some morphological characters take several months to appear. To date around 210 samples have been analysed; *P. chlamydospora, P. aleophilum*, *Cylindrocarpon spp.* and *Botryosphaeria*-like species have made up around 80% of the grapevine decline-associated fungi identified.

Results

Two multiplex PCR systems were developed that can identify over 80% of the fungi associated with grapevine decline in Castilla y León. Moreover, primers were designed for a TaqMan probe for use in a real-time PCR system that allowed *P. chlamydospora* to be quantified.

Multiplex-PCR

The first multiplex PCR system was developed, using the primers designed by Tegli *et al.* (2000), to detect *P. chlamydospora* and *P. aleophilum* at the same time in a single PCR reaction (annealing temperature 62ºC). The *P. chlamydospora* and *P. aleophilum* amplicons (380bp and 400bp respectively) were separated by 1.5% agarose gel electrophoresis and visualised via ethidium bromide staining. The second multiplex-PCR system detected *Botryosphaeria*-like and *Cylindrocarpon* species. The ITS sequence of the *Botryosphaeria*-like species allowed specific PCR primers to be designed. These were then used together with *Cylindrocarpon* spp.-specific primers (Fabritius *et al.*, 2006) to optimise the system. The reaction gave a *Botryosphaeria*-like species fragment of 395bp and *Cylindrocarpon* spp. amplicon of 317bp, both of which were detected as above. Both multiplex-PCR reactions were performed using purified DNA, spores, and pieces of decayed wood as templates.

Real-time PCR

Wiechel *et al.* (2005) designed a quantitative PCR system using a TaqMan probe to detect *P. chlamydospora* in nursery water samples during grapevine propagation. They amplified a product of 197bp. In the present study, a pair of primers and a TaqMan probe were designed, based on the ITS rDNA genes, to improve the specificity and sensitivity of *P. chlamydospora* detection. A product of 79bp was amplified using purified DNA and spores as a template. Less than 0.02ng/µl of DNA were detected with our primers when using purified DNA and less than 0.2spores/µl gave enough DNA to be detected under the experimental conditions employed.

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Detection of *Ralstonia solanacearum* from soil and water samples by the enrichment PCR method

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Ralstonia solanacearum is a soil-borne pathogen causing bacterial wilt on tomato and many other crops. The pathogen is able to survive in soil, possibly through weeds, and spreads in water. A simple detection method known as enriched-PCR (Er-PCR) was developed to detect the pathogen from soil and water samples. The method utilizes a selective medium, SM-1 (Tsai *et al.*, 1985), to multiply the pathogen before its detection by PCR using a species-specific primer pair, AU759/760 (Opina *et al.*, 1997). For soil samples, 10 gram of sample soils were added into a 250-ml flask containing 90 ml sterilized water then shaken at 180 rpm for 30 minutes. Then 1 ml of soil suspension was added into a 50-ml flask containing 9 ml of SM-1 broth, incubated at 30°C for three days and shaken at 160 rpm. The 25-µl reaction mixture used contained 1X PCR buffer, 1.5 mM $MgCl_2$, 0.05 mM of each dNTP, 1 pmol of primers, 2 unit of *Taq* DNA polymerase (Promega, Madison, USA), and 5 µl of bacterial suspension, soil suspension or water sample (previously boiled for 5 min). Using artificially infested soils prepared from AVRDC farm soil, sensitivity of the Er-PCR was determined to be 10 to 100 cells per gram of soil.

To compare sensitivity of Er-PCR with direct plating onto SM-1 plates, bulk soil, weed rhizosphere soil and irrigation water samples were collected from 10 fields in major tomato production areas in Taiwan (Table 1). Field soils were collected by dividing the field into 50 m^2 units. Nine sub-samples were collected from each unit along a zigzag transect and bulked. Weeds with attached soil were sampled randomly from among the predominant weed species in three fields, WL, SF and SS. Most soil was removed from the roots of these plants before rhizosphere soil was collected by shaking the roots in a 250-ml flask filled with 20 to 50 ml sterile water (depending on the root biomass). A total of five irrigation water samples containing 50 ml each were collected at the entrance point of each field. All these soil suspensions and water solutions were directly used to test for *R. solanacearum*.

Results showed that Er-PCR had better sensitivity than direct plating for 18 out of 19 tested cases (Table 1). In two fields, SS and CS, the pathogen was not detected by SM-1 plating, but was detected by Er-PCR with very high frequency. Such a low sensitivity of SM-1 could be due to the presence of diverse microorganisms, in large quantities, in the soil samples inhibiting *R. solanacearum* growth on the plates. Moreover, no history of bacterial wilt was reported for these two fields and they had not grown tomatoes for at least two seasons. This might have reduced the pathogen population present in the field to a very low level. The positive detections in weed and water samples from Field SS suggest the pathogen in Field SS was associated with weeds and introduced by irrigation water. Positive detection of the pathogen in weed rhizospheres occurred in nine out of the 11 collected species. This result confirms that weed rhizospheres are a common refuge for *R. solanacearum* and a means for it to survive. Field WL, SF and SS share a common irrigation water source from a near-by water reservoir and this water is distributed through canals. Drainage water commonly travels along the same canals thus moving the pathogen from infested fields. Therefore, contaminated water sources should be taken into account when designing local disease management strategies.

An experiment was conducted to determine the minimum number of soil samples needed to detect *R. solanacearum* in affected fields. Soil samples were collected from field units measuring 50, 100 and 200 m^2. This was done by sampling in 50 m^2 units and then mixing two or four samples into one. The number of positive samples was similar whatever the sampling area (Table 2). Therefore, five samples per field unit measuring 200 m^2 could be suggested as the minimum number for avoiding false negative *R. solanacearum* detections.

In conclusion, Er-PCR is a sensitive and efficient method to detect *R. solanacearum* from soil and water samples collected from production fields. This method could be used in ecological and epidemiological studies especially to predict potential risk of having bacterial wilt in a particular field. Considering the cost of PCR, detection could be conducted with SM-1 first and only negative samples would be re-analyzed with Er-PCR.

Table 1. Detection of *R. solanacearum* from field soils, weed rhizosphere soils and water at field entrance points collected from 10 vegetable fields

		Field soil		Weed rhizosphere soil		Water at entrance	
Field code[1]	BW[2]	SM-1	PCR	SM-1	PCR	SM-1	PCR
WL-110402	+	13/20[3]	20/20	2/11	8/11	0/5	1/5
WL-050603	+	13/20	17/20	1/15	5/15	0/5	0/5
SF-110502	-	17/20	17/20	4/31	23/31	0/5	4/5
SS-110402	-	0/20	8/20	1/12	8/12	0/5	2/5
TC1-061603	+	4/20	10/20	n.t.[4]	n.t.	n.t.	n.t.
TC2-102202	+	0/20	11/20	n.t.	n.t.	n.t.	n.t.
TC3-031502	-	13/20	18/20	n.t.	n.t.	n.t.	n.t.
TC4-121702	+	4/20	11/20	n.t.	n.t.	n.t.	n.t.
HS-040202	+	7/20	12/20	n.t.	n.t.	n.t.	n.t.
YC-072302	+	3/20	8/20	n.t.	n.t.	n.t.	n.t.
CS-070402	-	0/20	16/20	n.t.	n.t.	n.t.	n.t.

[1] Field codes mean location- month/day/year.

[2] History of bacterial wilt incidence in sampled field: + (incidence) and – (no incidence).

[3] Data presented are numbers of positive detections vs. total numbers of samples.

[4] n.t. – not tested.

Table 2. Detection of *R. solanacearum* in fields with different sampling unit areas

	SM-1			Er-PCR		
Field code	50 m^2	100 m^2	200 m^2	50 m^2	100 m^2	200 m^2
HS-040202	7/20[1]	2/10	1/5	12/20	5/10	3/5
CS-070402	0/20	0/10	0/5	16/20	10/10	4/5
YC-072302	3/20	1/10	1/5	8/20	6/10	2/5
WL-030403	15/20	8/10	4/5	19/20	9/10	5/5
TC1-061603	4/20	4/10	1/5	10/20	7/10	2/5

[1] Data presented are numbers of positive detections vs. total numbers of samples.

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