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Early warning model for cucumber downy mildew in unheated greenhouses

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Abstract An early warning model for occurrence of cucumber downy mildew in non-heated greenhouses was developed based on disease records and microclimatic parameter analysis. It also integrated empirical and fundamental forecasting models, early warning theory and plant protection product risk analysis. The thresholds for infection were a daily temperature range $\leq 5^{\circ}\text{C}$, daily mean RH $\geq 80\%$ and daily mean temperature at $15\text{--}25^{\circ}\text{C}$ in autumn. The temperature contribution rate for symptom appearance was measured by the reciprocal of the days of the inoculation period and the relationship was fitted by a polynomial of degree 3. Data recorded in two greenhouses were used to construct the model and its predictions were evaluated with an independent dataset. The warning source search, warning

obviation and plant protection production risk analysis were based on China's Good Agricultural Practices (CHINAGAP). The implications of the variables and modules of the model in the safety production are discussed.

Keywords cucumber downy mildew; early warning model; greenhouse; good agricultural practice (GAP); risk analysis

INTRODUCTION

Cucumber downy mildew, caused by *Pseudoperonospora cubensis* (Berk. & Curt.) Rostov. (Li et al. 2001; Shetty et al. 2002), is a major limitation to the production of greenhouse cucumbers. Means for predicting epidemic outbreaks are not currently available to most growers in China. The control of cucumber downy mildew depends on repeated fungicide sprays every 7 to 14 days or frequent application of fungicides when the symptoms appear. An early warning model that includes risk analysis and a related information system is needed to best respond to consumer concerns about food safety, environmental protection, worker health, safety and welfare, and production cost.

Early warning theory originated with the military, but its use has been extended into the economics of earthquake, flood, biological disaster areas. There is general agreement that early warning is addressed in five stages, confirming early warning index, analysis of early warning indicators, searching early warning sources, providing early warnings and warning situation obviation (Tao et al. 1994; Wu et al. 1999). Although there are many pest warning models, such as for processing tomatoes (Gleason et al. 1995), cotton bollworm (Bai et al. 2002), pear blossom blast (Latorre et al. 2002), few have involved early warning theory (Li 2007). Some cucumber disease early warning systems are not based on real-time environmental information (Xu 2003), and some need more support from accurate early warning models (Lu 2004; Ju 2006).

Infection of cucumbers by *P. cubensis* has been studied internationally for many years. A wide range of models have been developed more recently to forecast disease epidemics and most of these predict disease index (He et al. 2001; Xiong & Ji 2002; Xu 2003; Hua et al. 2005; Ju 2006). Of those predicting disease outbreaks, current models are of two kinds: empirical models (Kong et al. 1997; Li 2006); and fundamental models (Cohen 1977; Hong et al. 1989, 1990). Empirical models need more scientific and sound interpretation and fundamental models are difficult to apply because of their complex parameters. There is useful potential in aligning and integrating these two kinds of models and also in including other theories, such as those of early warning and risk analysis (FAO/WHO 1995, 1997, 1998). Based on China's Good Agricultural Practices (CHINAGAP, GB/T 20014.1–20014.5 2005), such an early warning model for cucumber downy mildew in unheated greenhouses was developed and the field validation for the model was conducted in Beijing, China.

MATERIALS AND METHODS

Cultural practices

This study was conducted in three unheated greenhouses located at Beijing Xiaotangshan Precision Agriculture Experimental Base, in Changping district, Beijing, PR China (40°10.6'N, 116°26.3'E) in 2006 and 2007. The site is in a warm temperate zone with a mean annual rainfall of 507.7 mm and a mean annual temperature of 13°C. Each greenhouse was 30 × 7 m and constructed of metal arches covered with polyethylene films. The greenhouses (no. 1–3) were north-south orientated and in a row.

The disease-resistant cucumber (*Cucumis sativus* L.) "Jingyanmini No.2" (Li & Zhu 2005; Zhang et al. 2006a) was used in all experiments. The seedlings were planted on 1 October and transplanted on 13 October 2006 and the harvest season was finished on 23 January 2007. The crops were maintained according to the recommendations of the Beijing Vegetable Research Center (BVRC), China, with pesticides including fungicides for the control of downy mildew being applied as instructed. The cultural practices were recorded thoroughly for tracing the warning source.

Disease investigation

The inoculum was the natural pathogen of these greenhouses. Five groups of 15 plants in an

"X"-pattern in each greenhouse were used for disease observation. These groups were named South-west, North-west, Middle, South-east and North-east. Every investigation was conducted in the morning before the dew dried. After transplanting the seedlings, an extensive survey was carried out each day until the appearance of greenish infected tissues and the day of first occurrence was recorded. After that, a systematic survey was conducted every 2–3 days on all the leaves of each sample plant. A disease severity index was used as follows: 0 = 0%; 1 = 0–5%; 3 = 6–10%; 5 = 11–25%; 7 = 25–50%; 9 = 50–100% (GB/T 17980.26 2000).

Microclimatic information collection

Air temperature and relative humidity in the greenhouse were recorded every 30 min by sensors coupled to a datalogger, which was portable equipment for measuring temperature, relative humidity (RH) and dew point temperature (Zhang et al. 2006b). The sensors were located in the centre of each group of crops in the greenhouse and shielded from direct solar radiation by suitable shelters. At first, the sensors were 10 cm higher than the plants and moved upwards regularly as the plants grew. When the plants reached 1.5 m, the sensors were fixed within the central part of the canopies. The outside weather parameters, temperature, relative humidity and solar radiation were also recorded by an outside weather datalogger every day for 30 min interval. The data was transferred from the datalogger (RS485 connection) to an environmental control computer (RS232 connection) via a local area network (LAN) and stored in a Microsoft Access 2000 database. Analyses of sampling variance and standard deviation were done using SAS8.02. The figures were drawn using Microsoft Excel 2003.

RESULTS

Integrating plant disease epidemiology, early warning theory, risk analysis theory and the CHINAGAP standard, the flow chart of an early warning system for cucumber downy mildew is shown in Fig. 1.

Warning indicators analysis (infection model)

Warning indicators are the direct causes of disease occurrence. Because *P. cubensis* is a widely existing pathogen in cucumber production, and with modern agricultural techniques whereby cucurbits may be cultivated all year round, the primary inoculum sources could meet the requirements for infection.

Fig. 1 The model structure of early warning and risk analysis for cucumber downy mildew. **Table 1** The relationship of some warning source and leaf wetness duration/RH.

Warning source	Relationship of warning source and leaf wetness duration/RH	Measurements
Irrigation	Improper irrigation techniques, such as overhead sprinkling will increase dew formation. Sprinkling during the night time has been proved advantageous in relation to <i>P. cubensis</i> on cucumbers.	Choosing the proper techniques such as furrow or drop irrigation. The time of day at which sprinkling should be performed depends largely on the presence or absence of dew. The ventilation in time is required.
Successive overcast weather	It will force the growers to reduce the ventilation time to maintain the temperature, and the solar radiation is not effective, which results in the higher humidity and longer leaf wetness duration.	In this condition, uncovering the thermal insulating covering and ventilation could be conducted at noon for a few hours.

Also there are no obvious highly resistant varieties at present in China. So emphasis was placed on environmental and cultural factors and the ways in which they affected disease development.

Many environmental factors can influence infection rates, leaf wetness duration (W) and canopy temperature during the wet period (T) are the dominant variables that control infection (Cohen 1977; Madden & Ellis 1988; Hong et al. 1990; Shi et al. 2005). According to practical situation, the duration of leaf wetness was replaced by the surrogate, duration of $RH \geq 93\%$ (Zheng et al 1990). However,

wetness duration is also related to radiation and other parameters such as air flow, so the error of this simplistic assumption rises when relative humidity is low. So another combination of parameters, such as daily range of temperature, daily mean RH and temperature, should be established for a qualitative approach (Yunis et al. 1994) for predicting infection. The precision and predictive ability of the models were verified and validated. Verification was accomplished using the same datasets (no. 2 and 3 greenhouses) that had been used to construct the models (Fig. 2). Models that produced reasonable

Fig. 2 Daily mean temperature, RH and range of temperature curves to determine the downy mildew occurrence are recorded in no. 2 and 3 non-heated greenhouses in 2006–07. Vertical lines indicate the predicted time of infection. The horizontal dotted lines indicate the qualitative threshold values of microclimate parameters at that time. Results are means of 10 replicates. Bars indicate SE.

predictions were examined further, and validated with the no. 1 greenhouse dataset.

The microclimatic parameter curves were used to determine thresholds for infection. These thresholds were daily range of temperature $\leq 5^{\circ}\text{C}$, daily mean RH $\geq 80\%$ and daily mean temperature at $15\text{--}25^{\circ}\text{C}$ in autumn. At nos 2 and 3 greenhouses in 2006–07, this happened between days 7–8 after planting.

Warning indicators analysis (incubation period and symptom appearance model)

Symptom appearance of downy mildew depends more on temperature than on leaf wetness duration. Li (2006) concluded that 5–7 days between infection and symptom appearance at $15\text{--}16^{\circ}\text{C}$ per day compared with 4–5 days at $17\text{--}19^{\circ}\text{C}$, 3–4 days at $20\text{--}25^{\circ}\text{C}$ and 8–10 days at $>25^{\circ}\text{C}$ as well as $<15^{\circ}\text{C}$. According to the data in Fig. 2, the temperature contribution rate (C) for symptom appearance was measured by reciprocal of days of inoculation period and the relationship of daily mean temperature (T) and C was fitted using SAS and expressed as:

$$C = -0.00069T^3 + 0.03625T^2 - 0.59208T + 3.19865 \quad (R^2 = 0.9215, P = 0.0112) \quad (1)$$

Then the thresholds for symptom appearance is: after the incubation period, if the average temperature over the previous night meets the range of $15\text{--}20^{\circ}\text{C}$, and the average RH $\geq 83\%$, then the symptom will appear within 24 h.

Warning source searching

Warning source is the primary cause of warning indexes and direct cause of warning indicators. As the similar reason of selection of warning indicators, the outside weather and cultural practices are the main warning sources in this paper. Because the optimal temperature of cucumber production is similar to that for *P. cubensis* development and symptom production, the leaf wetness duration/RH is the domain parameter for downy mildew outbreak. The relationship of warning source and leaf wetness duration/RH is shown in Table 1 (Palti & Rotem 1981; Jarvis 1992). Referencing the table, when the warning indicators or warning indexes exceed the normal range, according to CHINAGAP standards, the warning sources could be detected in time.

Provide warning

After warning source search, the warnings are issued through an audio alarm and a light. The light uses a key with five disease classes (0, 1, 2, 3, 4) defined as green, blue, yellow, orange and red. The user can judge the warning based on a field survey, integrated greenhouse circumstances and their experiences. If they confirm the warning, the model will present warning obviation.

Warning obviation

According to different warning sources, the warning obviation measurements will be varied (see

Table 1). During the process of warning obviation, the users can input the real-time information and acquire change of disease-warning, which is helpful for decision-making. If the warning index is back to normal level, the white alert will be provided for users.

Risk assessment

All chemical plant protection products must be registered by the appropriate governmental body. If the user chooses the chemicals, these should all be suitable and justified (according to label recommendations or official registration body publication) for *P. cubensis*. For example, Chlorothalonil is for protecting cucumbers at an early stage and Propamocarb for curing at a later stage. The farmer must demonstrate that all pre-harvest withholding intervals have been observed for protection products applied to the crop. Specifically in continuous harvesting situations for cucumbers, there are systems in place in the field or greenhouses, e.g., warning signs, to ensure fail-safe compliance. Also, current documented evidence or records are available either for an annual plant protection product residue analysis for CHINAGAP registered product crops, or of participation in a third party plant protection product residue monitoring system which is traceable to the farm. For example, the maximum residue level (MRL) of Chlorothalonil on cucumbers must not exceed 1 mg/kg (NY 5074 2002).

Risk management

There should be a clear documented procedure for remedial steps and actions (this will include communication to customers, product tracking exercise) to be taken where a crop protection product residue analysis indicates an MRL (either for the country of production or for the countries where the produce is intended to be traded) is exceeded or the pre-harvest withholding interval is not compliant.

Risk communication

There is a complaint form available relating to issues of compliance with the CHINAGAP standard. This must be held on the farm, and available on request, a clearly identifiable document for complaints relating to issues of compliance with CHINAGAP. The complaints procedure ensures that complaints are adequately recorded, studied and followed up including a record of actions taken. There are documents of the actions taken with respect to such complaints regarding CHINAGAP standard deficiencies found in products or services.

Fig. 3 Daily mean temperature, RH and range of temperature curves to determine the downy mildew occurrence are recorded in no. 1 non-heated greenhouse in 2006–07. Vertical lines indicate the predicted time of infection. The horizontal dotted lines indicate the qualitative threshold values of microclimate parameters at that time. Results are means of 10 replicates. Bars indicate SE

Validation

The quantitative model was validated with the no. 1 greenhouse 2006–07 dataset. The infection occurred in day 8 (Fig. 3) and downy mildew symptom appears in day 14. The warning source was the overcast weather on the day after planting. The occasion was accurately predicted by the model (Table 2).

Table 2 Validation results of no.1 greenhouse in 2006–07.

Site	Sum of temperature contribution rate in 12th day	Sum of temperature contribution rate in 13th day	Predicted appearance day	Practical appearance day
South-west	0.92	1.21	14	14
North-west	0.91	1.20	14	14
Middle	0.92	1.20	14	14
South-east	0.90	1.19	14	14
North-east	0.94	1.20	14	14

DISCUSSION

This research investigated the empirical and fundamental strategy integration and establishment of early warning model of cucumber downy mildew for unheated greenhouses in China. Because the model will serve the practical needs of production as well as forming the basis of an early-warning information system, parameter acquisition and calculation should meet the actual situation in China. Real-time and continuous temperature and RH datasets are easier to obtain (Zhang et al. 2006) than leaf wetness duration at present, and there are many empirical models for reference (Kong et al. 1997; Li 2006), so the latter is practical for information system development. Hong et al. (1989, 1990) built the fundamental epidemiology model for cucumber downy mildew, but it seemed too complex to apply in practice. However, its component analysis method was the framework of infection model in this paper, which improved its characteristic of mechanism. When employing this integration method, an accurate, biologically sound prediction model was developed. The microclimatic parameter curves were used to determine thresholds for infection, which were daily range of temperature $\leq 5^{\circ}\text{C}$, daily mean RH $\geq 80\%$ and daily mean temperature at $15\text{--}25^{\circ}\text{C}$ in autumn. The development of the infection predicting model in non-heated cucumber greenhouses did not involve formal statistical analysis. Nevertheless, the reliability of the model may gain support from validation with an independent set of data. A validation procedure that involves visual comparisons, as here, can be much more critical than some statistical tests (Yunis 1994).

Field observations (Cohen 1977) showed that the initial appearance of the disease was dependent more on the proximity of a given field to a source of inoculum than on the prevailing temperature. However, *P. cubensis* is a widely existing pathogen in cucumber production fields, and with modern

agricultural techniques whereby cucurbits may be cultivated all the year round, so the question of primary inoculum sources seems of little practical importance. So it seems that temperature was the dominant factor affecting inoculation and symptom appearance (Li 2006). The temperature contribution rate (C) for symptom appearance was measured by reciprocal of days of inoculation period and the relationship of T and C was fitted. The occasion was accurately predicted by the model (Table 2).

It is not sufficient for only early warning infection, incubation period and symptom appearance to ensure the food safety, environmental protection, worker health, safety and welfare. The early warning, GAP and risk analysis theory provide new framework for disease cause analysis, prevention or cure and safety of ultimate product. Based on the archives management of cultural practices of GAP, the warning source could be found out and provide the goals of warning obviation. It is mainly due to the greenhouses with normally simple, unheated, wooden or metallic structures covered with plastic and with little or no mechanisation or automation in China. The topography and macroclimate outside the greenhouse clearly have great effects on the environment inside (Jarvis 1992). Plus the overcast weather, the environment in the greenhouse is generally warm, humid, and wind-free. This environment promotes the excellent growth of cucumbers, but it is also ideal for the development of diseases caused by bacteria and fungi. As for the warning obviation, to predict the efficiency of different kinds of moisture management, it has to be borne in mind that under natural conditions of high humidity throughout the night time in greenhouse little can be done to reduce the risk of downy mildew disease (Palti & Rotem 1981). However, under man-made conditions sub-optimal for the development of downy mildew in greenhouse, any practice designed to reduce the number of hours that tissues retain a water film, may

inhibit the disease development more decisively than any other practices. Within the course of warning obviation, the fungicide is indispensable sometime, so the ultimate food safety should rely on the plant protection product risk analysis for legal registration issues of chemicals, pre-harvest intervals and residue (Zhang 2006).

The model is applicable in over-winter cucumber of greenhouse in 2007 in China, but needs validation and verification at different seasons and sites, also the inoculum concentration should be added for the next step. Under the guideline of GAP and risk analysis, the early warning model could predict the occurrence trace, the warning source to decide the correct measurements and ensure the food safety.

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