

ORIGINAL RESEARCH ARTICLE

Application of intelligent spore capture system in prediction of cucumber downy mildew and cucumber powdery mildew

Shigang Gao¹, Jinyan Luo², Rong Zeng¹, Lihui Xu¹, Lei Chen², Fuming Dai^{1*}

¹ Shanghai Engineering Research Centre of Low-carbon Agriculture, Institute of Eco-Environment and Plant Protection, Shanghai Academy of Agricultural Sciences, Shanghai 201403, China. E-mail: fumingdai@163.com

² Shanghai Agricultural Technology Extension Center, Shanghai 201103, China.

ABSTRACT

In order to explore the application of the new integrated intelligent spore capture system developed in China in the prediction of cucumber downy mildew and cucumber powdery mildew, the main working parameters of the integrated intelligent spore capture system, such as the presence or absence of air cutting head, the height of air collection port and the time of air collection, were optimized by identifying the morphology of captured spores in the case of natural disease in the field. The relationship between the disease index of cucumber downy mildew and cucumber powdery mildew in greenhouse and the amount of spores captured was analyzed through the dynamic monitoring of disease and spores. The results show that when the air cutting head is not installed, the height of the air collection port is 70 cm, and the period of 10: 00–10: 30 was beneficial to the capture of spores. The disease index of cucumber downy mildew and cucumber powdery mildew had a strong positive correlation with the total amount of spores captured for 7 consecutive days. Continuous monitoring of cucumber downy mildew sporangia and rapid increase in the number is a predictor of the occurrence or rapid increase of cucumber downy mildew. The conidia of cucumber powdery mildew were not detected before the disease onset, and the number of conidia captured was still small at the peak of the disease. The research shows that the integrated intelligent spore capture system is suitable for the prediction of cucumber downy mildew, but there are still some problems in the prediction of cucumber powdery mildew.

Keywords: Cucumber; Downy Mildew; Powdery Mildew; Spore Capture; Forecast

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1. Introduction

Cucumber downy mildew, commonly known as Paomagan and Huolong, is caused by *Pseudoperonospora cubensis* (Berk. & Curt.) Rostov which is an airborne disease caused by infecting cucumber leaves and it spreads rapidly in cucumber cultivation areas under suitable climatic conditions. If the disease is not prevented and controlled in time in the early stage, it will cause serious yield reduction, or even no yield or harvest. It is a devastating disease on cucumber in the protected area^[1–3]. Cucumber powdery mildew, commonly known as white hair, also known as white hair disease and powdery mildew, is another airborne disease caused by the infection of *Podosphaera xanthii* (Castagne) V. P. Heluta^[4,5]. As long as the conditions are suitable, it can also quickly spread throughout the greenhouse in a few days and if not controlled in time, it will lead to scorched plant leaves, resulting in slow early growth of the fruit and premature plant aging, which will seriously affect the quality and yield of cucumber^[6]. The early prevention and control of the disease is very important to the occurrence and control of

the disease. Therefore, it is of great significance to accurately and quickly predict the downy mildew of cucumber and powdery mildew of cucumber before the disease occurs.

As for the forecast of downy mildew and powdery mildew, domestic research is mainly focus on building various forecast models through historical meteorological factors^[7-9]. Not only historical meteorological data but also accurate historical disease occurrence are required, and the establishment and use of forecast models require high personnel quality. Downy mildew and powdery mildew are air borne diseases, and their pathogenic spores spread with the air flow is the main reason for the occurrence and prevalence of the disease. Therefore, the number of pathogenic spores in the air is closely related to the occurrence and prevalence of the disease. Using spore capture technology to capture pathogenic spores in the air can provide basic data for the prediction of downy mildew and powdery mildew^[10,11]. At present, Neufeld *et al.*^[12] and Granke *et al.*^[13] believe that the sporangium of downy mildew of cucumber is correlated with environmental conditions and disease severity, which can be used to predict the transmission risk of downy mildew of cucumber. However, there is no report on the correlation between sporangium of downy mildew of cucumber and disease severity in China. In terms of the prediction and prediction of powdery mildew, it is mainly focused on wheat powdery mildew in China. Zhou *et al.*^[14] and Yao^[15] respectively used the imported mobile and constant volume spore catchers to monitor and predict wheat powdery mildew, while the research on the prediction of cucumber powdery mildew has not been reported. Mobile and constant volume spore catchers introduced from abroad capture spores in the air through capturing Petri dishes or capturing belts, but they still need a lot of time for in vivo culture or microscopic examination of pathogenic spores. In China, the rotary electric aerial spore catcher has been used for a long time to capture the spores of wheat scab in the air. However, this catcher needs to take slides every day and carry out indoor microscopic examination for identification and counting, which is time-consuming and la-

bor-consuming, with large human error.

In this study, the newly developed integrated intelligent spore capture system with the functions of automatic spore capture, automatic micrograph and automatic picture upload was used to capture and monitor the number of conidia of downy mildew fungus and powdery mildew of cucumber, and analyze the relationship between the number of spores and the occurrence of downy mildew and powdery mildew of cucumber, in order to provide technical basis for the spore capture system in the prediction of field diseases, and provide a new method to predict the occurrence of cucumber downy mildew and cucumber powdery mildew.

2. Materials and methods

2.1 Materials

Tested plants and varieties: the cucumber varieties Shenqing No.1 and Chunqiuwang, which are mainly planted in the suburbs of Shanghai and are susceptible to cucumber downy mildew and cucumber powdery mildew, were provided by Shanghai Funong Seed Co., Ltd. and the Institute of Protected Horticulture of Shanghai Academy of Agricultural Sciences.

Test instrument: DM 2000 microscope (Leica company, Germany); CT-BB-02 integrated intelligent spore capture system, Shanghai Gaolan Scientific Instrument Co., Ltd. Main technical parameters of the system: 65 cm long × 24 cm wide × 78 cm height; weight: 27 kg; height of air collection port: 65–150 cm; air collection flow: 0–400 L/min; duration of air collection: 1–100 min. Collect air samples through air collection port; automatically take microscopic pictures of the central recessed 8 mm × 54 mm collection area of the 25 mm × 60 mm spore collection slides of the integrated intelligent spore capture system by moving the lens, take 30 microscopic pictures continuously and store them in the system, and then upload the pictures to the management platform of the monitoring system via 3G or LAN network.

2.2 Method

2.2.1 Spore identification

The morphological changes of spores under the in vitro condition of artificial simulation: spore recognition is the key link of all spore traps for disease prediction. The sporangia of downy mildew of cucumber and the conidia of powdery mildew of cucumber released into the air may have morphological changes due to water loss, which is easy to cause misjudgment when identifying the morphology of the spores captured by the integrated intelligent spore capture system, resulting in inaccurate statistical data of spore number. Therefore, it is necessary to artificially simulate the in vitro conditions of the sporangium of downy mildew of cucumber and the conidia of powdery mildew of cucumber to determine their morphological characteristics at different times after being separated from the leaves of cucumber. Specific methods: a thin layer of Vaseline was evenly coated on ordinary glass slides, and the glass slides coated with Vaseline were gently adhered to the sporangia on the naturally occurring cucumber downy mildew infected leaves; gently shake off the conidia of cucumber powdery mildew and make it fall on another glass slide coated with Vaseline; the glass slides with sporangia of downy mildew of cucumber and conidia of powdery mildew of cucumber were placed in a 9 cm diameter glass Petri dish and cultured in a 25 °C constant temperature light incubator. The morphological changes of sporangia of downy mildew of cucumber and conidia of powdery mildew of cucumber were observed under a microscope after 0, 24, 96, 408 hours and 0, 1, 24, 408 hours, respectively.

Identification of naturally captured spores: download the micrograph of the spores captured by the integrated intelligent spore capture system from the monitoring system management platform, and identify the sporangia of cucumber downy mildew and conidia of cucumber powdery mildew on the micrograph according to the morphological characteristics of the spores of cucumber downy mildew and cucumber powdery mildew under artificial simulated in vitro conditions.

2.2.2 Parameter optimization test of integrated intelligent spore capture system

The air cutting head, air collection port height and air collection time of the integrated intelligent spore capture system have a great impact on the number of spores captured, while the cutting head has a great impact on the background impurities. Therefore, it is necessary to analyze the impact of the air cutting head, air collection port height and air collection time on the number of spores captured and the impact of the cutting head on the background impurities to determine whether to install the air cutting head, appropriate air collection port height and air collection time.

In the autumn of 2014, the optimization test was carried out in the greenhouse of the Zhuanghang Comprehensive Test Station of Shanghai Academy of Agricultural Sciences. The greenhouse was 40 m long and 8 m wide, with 4 borders, 38 m long, 0.8 m wide and 1.5 m apart. In the same greenhouse, Shenqing No.1 and the Chunqiuwang are all 2 plots and each plot has two rows of seeds, with a length of 36 m, a row spacing of 0.5 m, and a plant spacing of 0.5 m. The planting area of both varieties is 83.6 m². When the cucumber grows to 3–4 true leaves, two integrated intelligent spore capture systems are installed side by side in the center of the same cucumber greenhouse.

Determination of whether there is air cutting head or not: two integrated intelligent spore capture systems are respectively equipped with or without air cutting head, the height of air collection port is 150 cm, and the air collection time is 10:00–10:30, the sampling time lasts for 30 min, and the air collection flow is 80 L/min. Download the micrographs of spores captured by two integrated intelligent spore capture systems on September 9, September 11 and September 15, respectively, identify the sporangia of downy mildew of cucumber and conidia of powdery mildew of cucumber on the micrographs, count the number, and analyze the impact of air cutting head on the number of spores captured and the background impurities.

Optimization of air collection port height: two integrated intelligent spore capture systems are not equipped with air cutting heads. The air collection

port height is set to 70 cm and 150 cm respectively, and the air collection time is 10: 00–10: 30, the sampling time lasts for 30 min, and the air collection flow is 80 L/min. Download the micrographs of spores captured by two integrated intelligent spore capture systems on September 9, September 11 and September 15, respectively, identify the sporangia of downy mildew of cucumber and conidia of powdery mildew of cucumber on the micrographs, count the number, and analyze the impact of the height of air collection port on the number of spores captured.

Optimization of air collection time: two integrated intelligent spore capture systems are not equipped with air cutting heads. The height of the air collection port is 70 cm, and the air collection time is set to 04: 00–04: 30, 10: 00–10: 30, 13: 30–14: 00, 16: 00–16: 30 and 22: 00–22: 30 with each sampling time lasts for 30 min. The air collection flow is the same as above. Download the micrographs of spores captured by two integrated intelligent spore capture systems from April 10 to May 17, identify the sporangia of downy mildew of cucumber and conidia of powdery mildew of cucumber on the micrographs, count the number, and analyze the impact of air collection time on the number of spores captured.

2.2.3 Dynamic monitoring of disease occurrence and spore capture

The dynamic monitoring of the tested greenhouse and planting conditions are the same as 2.2.2. In the spring and autumn of 2015, the occurrence of cucumber downy mildew and cucumber powdery mildew and the captured spores were dynamically monitored. The dynamic monitoring time in the spring and autumn was from March 30 to May 18 and August 31 to October 12, respectively. When the cucumber grows to 3–4 true leaves, an integrated intelligent spore capture system is placed in the center of the greenhouse, and the parameters optimized in 2.2.2 are used for dynamic monitoring.

The occurrence of cucumber downy mildew and cucumber powdery mildew were regularly observed on April 24, April 30, May 7, May 14, September 23, September 30 and October 7, respectively. The incidence levels of the two diseases were

investigated and counted, and the disease index was calculated. Investigation and expression method of incidence level: three fixed investigation areas are set up for each of the two varieties, and all leaves of five plants with normal growth are investigated at fixed points in each area, expressed as the percentage of disease spot area of each leaf in the whole leaf area. The degree of cucumber downy mildew is classified according to GB/T 17980.26-2000: grade 0: no disease spot; grade 1: the lesion area accounts for 5% or less of the whole leaf area; grade 3: the lesion area accounts for 6%–10% of the whole leaf area; grade 5: the lesion area accounts for 11%–25% of the whole leaf area; grade 7: the lesion area accounts for 26%–50% of the whole leaf area; grade 9: the lesion area accounts for more than 50% of the whole leaf area. The degree of cucumber powdery mildew is classified according to GB/T 17980.30-2000, grade 0: no disease spot; grade 1: the lesion area accounts for 5% or less of the whole leaf area; grade 3: the lesion area accounts for 6%–10% of the whole leaf area; grade 5: the lesion area accounts for 11%–25% of the whole leaf area; grade 7: the lesion area accounts for 21%–40% of the whole leaf area; grade 9: the lesion area accounts for more than 40% of the whole leaf area. Disease index = \sum (Number of diseased leaves at all levels \times Relative order value)/(Total number of leaves investigated \times 9) \times 100.

Download the micrographs of spores captured from March 30 to May 18 and from August 31 to October 12, identify the sporangia of downy mildew of cucumber and the conidia of powdery mildew of cucumber on the micrographs, and count the number of spores captured each day. Calculate the total number of sporangia of downy mildew of cucumber and conidia of powdery mildew of cucumber captured on the day of disease investigation and the first 6 days respectively, and analyze the correlation between the number of sporangia captured on the 7 days and the disease index. The correlation coefficient R is greater than 0 for positive correlation, less than 0 for negative correlation, $0 < |R| \leq 0.09$ for non-correlation, $0.1 \leq |R| < 0.3$ for weak correlation, $0.3 \leq |R| < 0.5$ for medium correlation, $0.5 \leq |R| \leq 1.0$ for strong correlation.

2.3 Data analysis

The Microsoft Excel 2010 software was used for correlation analysis and *t*-test difference significance test.

3. Results and analysis

3.1 Spore identification

3.1.1 Morphological changes of spores under artificial simulated in vitro conditions

After 0, 24, 96 and 408 hours on a glass slide coated with Vaseline, the shape of the fresh sporangium of cucumber downy mildew naturally occurred changed slightly, most of which were olive shaped, dark brown, and seemed to have depressions or cracks in the middle (**Figure 1A–D**). The conidia of naturally occurred cucumber powdery mildew were initially oval and light colored on a glass slide coated with Vaseline, and the cells were full of protoplasm (**Figure 1E**). However, after 1 h of in vitro, the protoplasm gathered to the two ends of the cell, the center of the cell became empty and transparent, and the spores began to deform (**Figure 1F**); after 24 hours of in vitro, the conidia were basically hollow and transparent (**Figure 1G**); after 408 h of in vitro, the conidia were completely hollow (**Figure 1H**).

3.1.2 Identification of naturally captured spores

According to the morphological changes of sporangium of downy mildew of cucumber and conidia of powdery mildew of cucumber under simulated in vitro conditions, the sporangium of downy

mildew of cucumber and conidia of powdery mildew of cucumber were identified on the micrograph of captured spores (**Figure 2**).

The morphology of the captured sporangium and conidia are basically the same as that of the newly collected sporangium of downy mildew of cucumber and the conidia of powdery mildew of cucumber from the natural disease samples (**Figure 2A**). Therefore, the two captured spores are identified as the sporangium of downy mildew of cucumber and the conidia of powdery mildew of cucumber respectively; the morphology of the captured conidia is similar to that of the conidia of powdery mildew of cucumber whose morphology changes under artificial in vitro conditions (**Figure 2B**). Therefore, the captured conidia are identified as the conidia of powdery mildew of cucumber. In addition to the target pathogen spores to be monitored, the integrated intelligent spore capture system also captured *Alternaria spp.* (**Figure 2C**) and some other fungi (**Figure 2D**). These spores are quite different in morphology from downy mildew sporangia and cucumber powdery mildew conidia, and are easy to distinguish.

3.2 Optimization of parameters of integrated intelligent spore capture system

3.2.1 Determination of with/without air cutting head

The installation of cutting head in the integrated intelligent spore capture system can significantly reduce the amount of particulate impurities in the collected air, but also eliminate the target

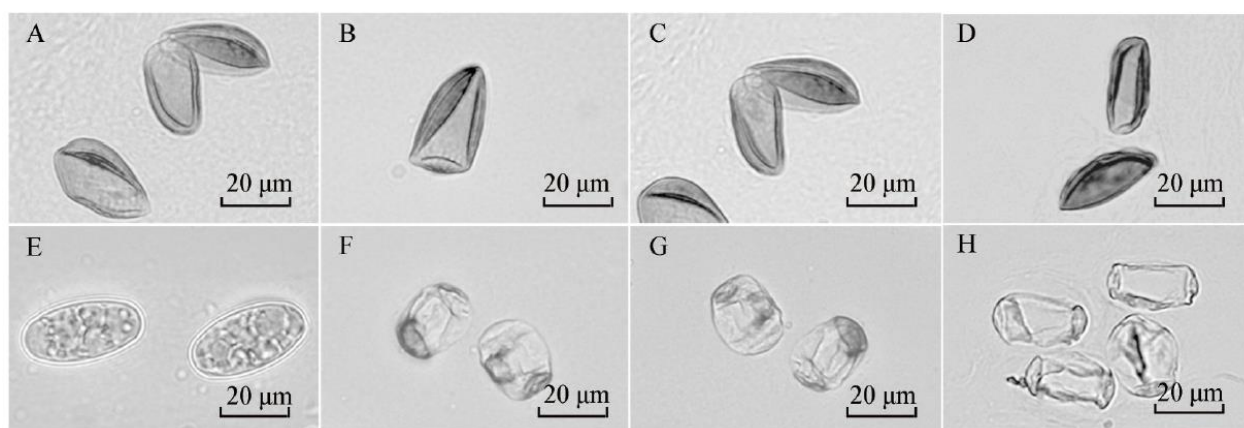


Figure 1. Morphological change of sporangia of *Pseudoperonospora cubensis* and conidia of *Podosphaera xanthii* in vitro. A–D: Sporangium morphology at 0, 24, 96 and 408 h, respectively; E–H: conidium morphology at 0, 1, 24 and 408 h, respectively.

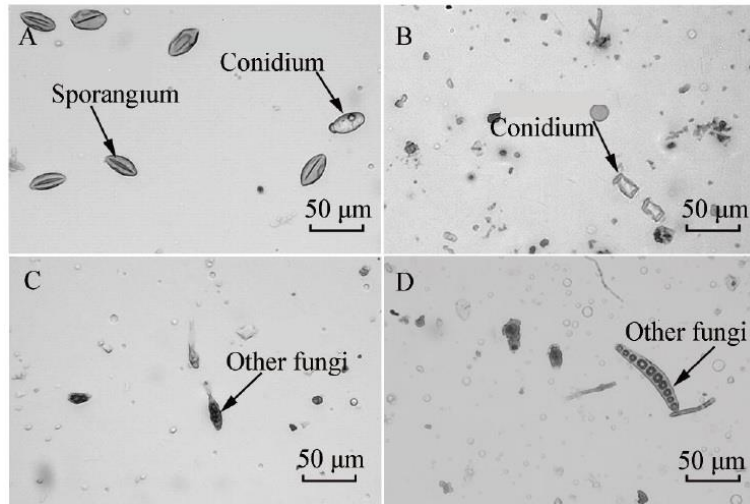


Figure 2. Morphology of spores trapped by automatic spore-trap and identification system.

A: Sporangia of *P. cubensis* and conidia of *P. xanthii* with normal morphology; B: conidia of *P. Xanthii* with abnormal morphology; C, D: conidia of other fungi.

pathogen spores in the collected air. The sporangium of downy mildew of yellow melon and conidia of powdery mildew of yellow melon cannot be observed on the spore collection slide (Table 1). Therefore, it is determined that the integrated intelligent spore capture system will not be installed with cutting head.

3.2.2 Optimization of air collection port height

When the height of the air collection port of the integrated intelligent spore capture system is 70 cm, the number of sporangia of cucumber downy mildew is significantly higher than that of the air collection port, which is 150 cm, while the number of conidia of cucumber powdery mildew is slightly increased (Table 1). Therefore, the height of the air collection port should be 70 cm.

Table 1. Determination of the use of panicle separator and optimization of air-collecting height in automatic spore-trap and identification system

| Parameter optimization | With/without air panicle separator | Air-collecting height (cm) | 2014-09-09 | | 2014-09-11 | | 2014-09-15 | |
|---------------------------------------|------------------------------------|----------------------------|------------|---|------------|---|------------|---|
| | | | A | B | A | B | A | B |
| With/without panicle separator | With | 150 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Without | 150 | 1 | 2 | 2 | 1 | 4 | 0 |
| Optimization of air-collecting height | Without | 70 | 3 | 3 | 7 | 2 | 13 | 3 |
| | Without | 150 | 1 | 2 | 2 | 1 | 4 | 0 |

A: *P. cubensis*; B: *P. xanthii*.

3.2.3 Optimization of air collection time

The number of sporangia of cucumber downy mildew monitored by the integrated intelligent spore capture system was significantly higher ($P < 0.01$) in the air collection time of 10:00–10:30 than in other times; the number of conidia of cucumber powdery mildew monitored was extremely significantly higher ($P < 0.01$) in the 04:00–04:30, 16:00–16:30 and significantly higher in the time periods if 22:00–22:30 ($P < 0.05$) and 22:00–22:30 ($P < 0.05$), but there is no significant difference com-

pared with the time period of 13:30–14:00 (Table 2). Although there was no significant difference in the number of cucumber powdery mildew conidia monitored in the air collection time periods of 10:00–10:30 and 13:30–14:00, the number of days that conidia were detected was 16 days, higher than that of 10 days in the period of 10:00–10:30. Therefore, the best air collection time for monitoring cucumber downy mildew and cucumber powdery mildew was determined to be 10:00–10:30 (Table 2).

Table 2. Optimization of air-collecting time with automatic spore-trap and identification system

| Date | Amount of trapped spores | | | | | | | | | |
|------------|--------------------------|---------|-------------|----|-------------|-------|-------------|---------|-------------|--------|
| | 04:00–04:30 | | 10:00–10:30 | | 13:30–14:00 | | 16:00–16:40 | | 22:00–23:30 | |
| | A | B | A | B | A | B | A | B | A | B |
| 2015-04-10 | 0 | 0 | 10 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2015-04-11 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2015-04-12 | 0 | 0 | 9 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2015-04-13 | 0 | 0 | 12 | 3 | 0 | 0 | 0 | 1 | 0 | 1 |
| 2015-04-14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2015-04-15 | 0 | 0 | 9 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 2015-04-16 | 0 | 0 | 8 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| 2015-04-17 | 0 | 0 | 4 | 0 | 1 | 3 | 0 | 0 | 0 | 0 |
| 2015-04-18 | 0 | 0 | 67 | 5 | 1 | 1 | 0 | 0 | 0 | 0 |
| 2015-04-19 | 0 | 0 | 185 | 0 | 6 | 0 | 0 | 0 | 0 | 0 |
| 2015-04-20 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 |
| 2015-04-21 | 0 | 0 | 2 | 0 | 0 | 11 | 0 | 0 | 0 | 0 |
| 2015-04-22 | 0 | 0 | 21 | 1 | 4 | 0 | 0 | 0 | 0 | 0 |
| 2015-04-23 | 0 | 0 | 5 | 10 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2015-04-24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2015-04-25 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2015-04-26 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 |
| 2015-04-27 | 0 | 0 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2015-04-28 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 2015-04-29 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2015-04-30 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2015-05-01 | 0 | 0 | 3 | 1 | 0 | 1 | 0 | 0 | 0 | 0 |
| 2015-05-02 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2015-05-03 | 0 | 0 | 3 | 3 | 1 | 1 | 0 | 0 | 0 | 0 |
| 2015-05-04 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2015-05-05 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2015-05-06 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2015-05-07 | 0 | 0 | 72 | 1 | 3 | 0 | 0 | 0 | 0 | 0 |
| 2015-05-08 | 0 | 0 | 34 | 4 | 1 | 11 | 0 | 0 | 0 | 0 |
| 2015-05-09 | 0 | 0 | 26 | 0 | 23 | 0 | 0 | 0 | 0 | 3 |
| 2015-05-10 | 0 | 0 | 167 | 19 | 4 | 1 | 0 | 0 | 0 | 0 |
| 2015-05-11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2015-05-12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2015-05-13 | 0 | 0 | 0 | 0 | 3 | 1 | 0 | 0 | 0 | 0 |
| 2015-05-14 | 0 | 0 | 46 | 1 | 1 | 5 | 1 | 1 | 0 | 0 |
| 2015-05-15 | 0 | 0 | 243 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| 2015-05-16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2015-05-17 | 0 | 0 | 102 | 5 | 13 | 13 | 2 | 0 | 0 | 0 |
| <i>P</i> | 0.005** | 0.007** | - | - | 0.008** | 0.614 | 0.005** | 0.008** | 0.005** | 0.011* |

A: sporangia of *P. cubensis*; B: conidia of *P. xanthii*; significant difference analysis was performed among amounts of spores trapped in different time points using *t*-test of Microsoft Excel 2010 Software; **: indicates of trapped downy mildew sporangia or powdery mildew conidia was extremely significantly less in 04: 00–04: 30, 13: 30–14: 00, 16: 00–16: 30 and 22: 00–22: 30 than in 10: 00–10: 30; *: indicates amount of trapped powdery mildew conidia in 22: 00–22: 30 was significantly less than in 10: 00–10: 30; -: no data.

3.3 Dynamic monitoring of cucumber downy mildew and capture sporangia

In the spring of 2015, about one week before the sporadic occurrence of cucumber downy mildew, the sporangia of cucumber downy mildew were continuously monitored, and the number of spores reached a peak of 202 near the onset of the disease; subsequently, the disease index (<0.8) and the number of sporangia monitored remained at a very low level; at the late stage of the disease, the disease index rose rapidly to 44.41, and the number of sporangia of downy mildew of cucumber began to

increase, and the second peak appeared (**Figure 3A**). The total amount of sporangia captured for 7 consecutive days was positively correlated with the disease index, and the correlation coefficient was $R = 0.5022$, which was a strong positive correlation (**Table 3**). In the autumn of 2015, about one week before the sporadic occurrence of cucumber downy mildew, a small number of sporangia were successively detected; subsequently, the disease index and the number of sporangia detected gradually increased, and the number of sporangia detected reached a peak of 1,561 before the disease index reached 41.72 in the late stage of the disease (**Fig-**

ure 3B). The total amount of sporangia captured for 7 consecutive days was positively correlated with the disease index, and the correlation coefficient was $R = 0.9275$, which was a strong positive correlation (Table 3). The above results showed that the disease index of cucumber downy mildew had a

strong positive correlation with the total amount of sporangia captured for 7 days ($R > 0.5$); the number of sporangia of cucumber downy mildew was detected for many days and the rapid increase was a predictive index for the occurrence or rapid increase of cucumber downy mildew.

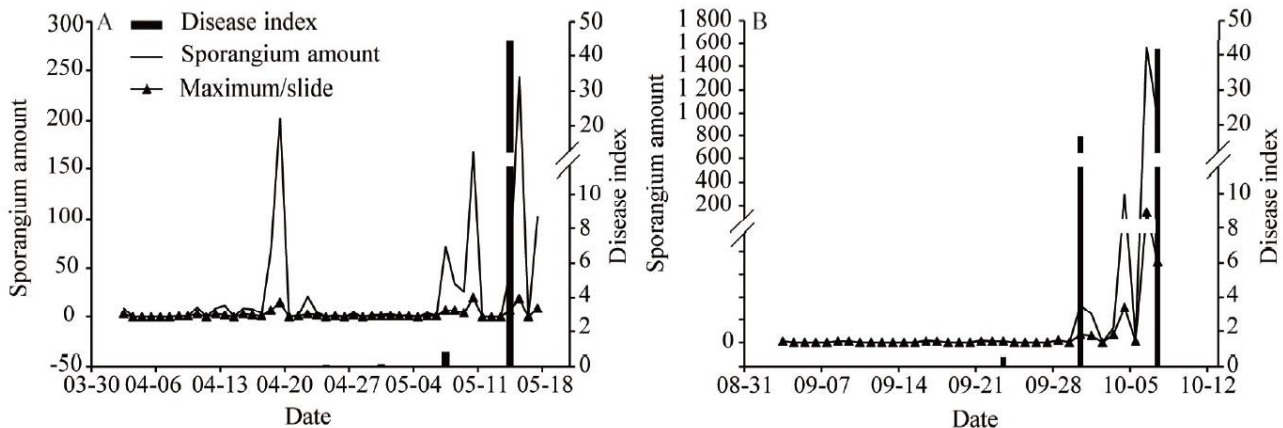


Figure 3. Dynamic monitoring of trapped sporangium amount and disease index of cucumber downy mildew in high-tunnel in the spring (A) and autumn (B) of 2015.

Table 3. Correlation analysis between the number of trapped spores and disease index

| Date | Cucumber downy mildew | | | Cucumber powdery mildew | | |
|-------|-----------------------|---------------|-------------------|-------------------------|---------------|-------------------|
| | Amount of sporangia | Disease index | Correlation index | Amount of conidia | Disease index | Correlation index |
| 04-10 | - | - | - | 2 | 0.37 | - |
| 04-17 | - | - | - | 7 | 0.84 | - |
| 04-24 | 297 | 0.11 | - | 16 | 4.47 | - |
| 04-30 | 10 | 0.15 | - | 1 | 19.08 | - |
| 05-07 | 83 | 0.80 | - | 8 | 41.57 | - |
| 05-14 | 273 | 44.41 | - | 24 | 49.64 | - |
| | | | 0.5022 | | | 0.5244 |
| 09-23 | 5 | 0.56 | - | - | - | - |
| 09-30 | 35 | 16.43 | - | - | - | - |
| 10-07 | 2 858 | 41.72 | - | - | - | - |
| | | | 0.9275 | | | |

Amounts of trapped sporangia or conidia are the sum of sporangia or conidia trapped in seven consecutive days containing date of disease survey; -: no data.

3.4 Dynamic monitoring of cucumber powdery mildew and capture of conidia

In the spring of 2015, the disease index gradually increased from the sporadic occurrence of cucumber powdery mildew to the late stage of the disease. The conidia of cucumber powdery mildew were not detected before the disease; in the initial disease stage, the number of conidia of cucumber powdery mildew increased with the increase of disease index; during the disease peak period, the dynamic change trend of the number of conidia of cucumber powdery mildew was basically consistent with the dynamic change trend of disease index, but the number of conidia did not increase significantly,

up to less than 20 (Figure 4). The total amount of conidia captured for 7 consecutive days was positively correlated with the disease index, and the correlation coefficient was $R = 0.5244$, which was a strong positive correlation (Table 3). In the autumn of 2015, the incidence of cucumber powdery mildew was very mild, with the highest disease index of 3.45, and no conidia of cucumber powdery mildew were detected in the same period. The above results show that: although the disease index of cucumber powdery mildew has a strong positive correlation with the total amount of conidia captured for 7 consecutive days ($R > 0.5$), the conidia of powdery mildew were not detected before the disease, and the amount of conidia captured during the

disease peak period is still small. Therefore, there are some problems in the application of the integrated intelligent spore capture system in the prediction of cucumber powdery mildew.

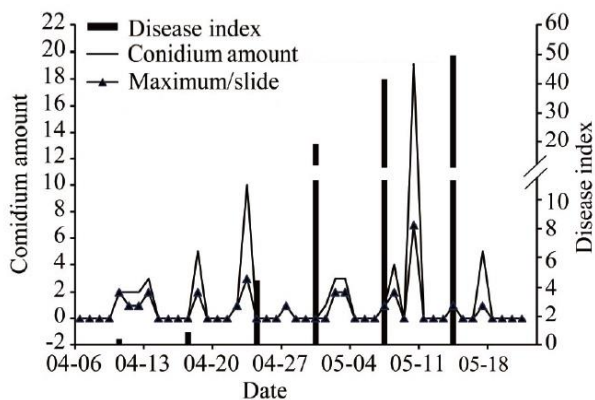


Figure 4. Relationships between trapped conidium amount and occurrence degree of cucumber powdery mildew in high tunnel in the spring of 2015.

4. Discussion

Spore morphology recognition is the key of spore capture technology. In this study, it was found that the morphology of conidia of cucumber powdery mildew change quickly under in vitro condition, while the morphology of sporangium of cucumber downy mildew was relatively stable. These results were very important for spore identification and accurate counting, because the integrated intelligent spore capture system also captured the deformed conidia of cucumber powdery mildew in the field application. In addition to morphological identification, a ruler bar is added to the automatically taken micrograph to identify the spores according to the spore size, so as to further improve the identification ability of pathogenic spores. The interference of impurities on the capture slide is also an important factor affecting spore recognition. The amount of impurities may be related to the wind, air flow and dust in the air of the capture area, because sometimes there are very few impurities and sometimes more impurities in the photos. In practical application, we try to remove or reduce the amount of impurities by adding cutting heads. Although the effect of removing impurities is obvious, the number of spores captured is significantly reduced. In addition, the soil surface in the area where the integrated intelligent spore capture system is installed is

covered with film to reduce the impurity particles in the air of the adjacent area.

The number of pathogenic spores in the air is closely related to the degree of disease^[16]. Zhao and Liu^[17] made it clear that the disease index of ginseng blackboard disease is highly positively correlated with the number of spores captured ($R = 0.8722$). Liu *et al.*^[18] found that the disease index of wheat powdery mildew in the field is significantly correlated with the concentration of conidia of powdery mildew in the air, which is consistent with the strong correlation between the disease index of cucumber downy mildew and cucumber powdery mildew and the number of spores captured in this experiment. Zhou *et al.*^[14] used a mobile spore catcher to effectively capture the conidia of wheat powdery mildew at the peak of the disease, but failed to capture the spores at the initial stage of the disease, which is different from the results of this test. In this test, the conidia can be captured in the facility shed through the integrated intelligent spore capture system, but the conidia were not captured before the disease of cucumber powdery mildew. With the aggravation of the disease, the number of spores captured has also increased, but the number of conidia captured is still low even at the peak of the disease. Compared with cucumber downy mildew, the number captured under the similar disease index is also 1–2 orders of magnitude lower. Therefore, the integrated intelligent spore capture system is more effective in capturing the sporangium of cucumber downy mildew.

The integrated intelligent spore capture system can automatically capture spores and upload micrographs. It can save time and labor in monitoring spores, and will have a good application prospect in the prediction of cucumber downy mildew. The integrated intelligent spore capture system is equipped with a microscope that automatically takes pictures. The clarity of the pictures taken is an important factor affecting spore recognition. Through observation and analysis, the thickness and flatness of the adhesive layer on the captured glass slide, as well as the number and size of impurity particles, will interfere with the microscopic focusing of the target pathogen spores and affect the

shooting effect. Therefore, in the future, it is necessary to continue to improve the flatness of the adhesive layer on the glass slide and the control of the number of impurities, so as to further improve the clarity of the photos.

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Conflict of interest

The authors declare that they have no conflict of interest.

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