

**INVASIVE ALIEN SPECIES AND THE IPPC,**  
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# **Speed Detection of Pests in China**

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Phytopathology falls back on technology. Today, with the rapid development of science, it is necessary that we enhance study level and make diagnosis more sensitive and specific. So we have done some researches on how to advance the diagnostic measures and methods in recent ten years.

# outline

- ◆ The general pest detection measures and methods in China
- ◆ Researching on the speed detection for phytosanitary
- ◆ The speed detection for pests of citrus
- ◆ Application of the speed detection in China

# 1 The general pest detection measures and methods in China

## ◆ 1.1 Detection by microscope (morphological method)

General pest detection measures are used to detect fungi and adult insect. Because the development of the taxonomy and appearance of new race and variety, the morphological method is restricted in many aspects such as the identification of wheat dwarf bunt (*Tilletia controversa*), wheat karnal bunt (*T. indica*), and wheat common bunt (*T. tritici*). It is hard to detecting seed-brought pests using the morphological method.



## 1.2 Isolation culture

Isolation culture is an assistant measure to detect fungi and bacteria. It is an effective method to detecting seed-brought disease or system infection disease. But it has to take a long time more than 1~2 weeks.

### ◆ 1.3 Inoculating the indicated plant

This method is mainly used to the virus disease. Before the detection methods by immunology and molecular biology come into being, we have to fall back on inoculating the indicated plant and spend a long time in detection. Virus are very important for young plants, we must search the effective and speed methods for detection immediately.

*Cyphomandra betacea*



Monroe bean

The symptom of the indicated plant detecting TRSV

## 2. Researching on the speed detection for phytosanitary

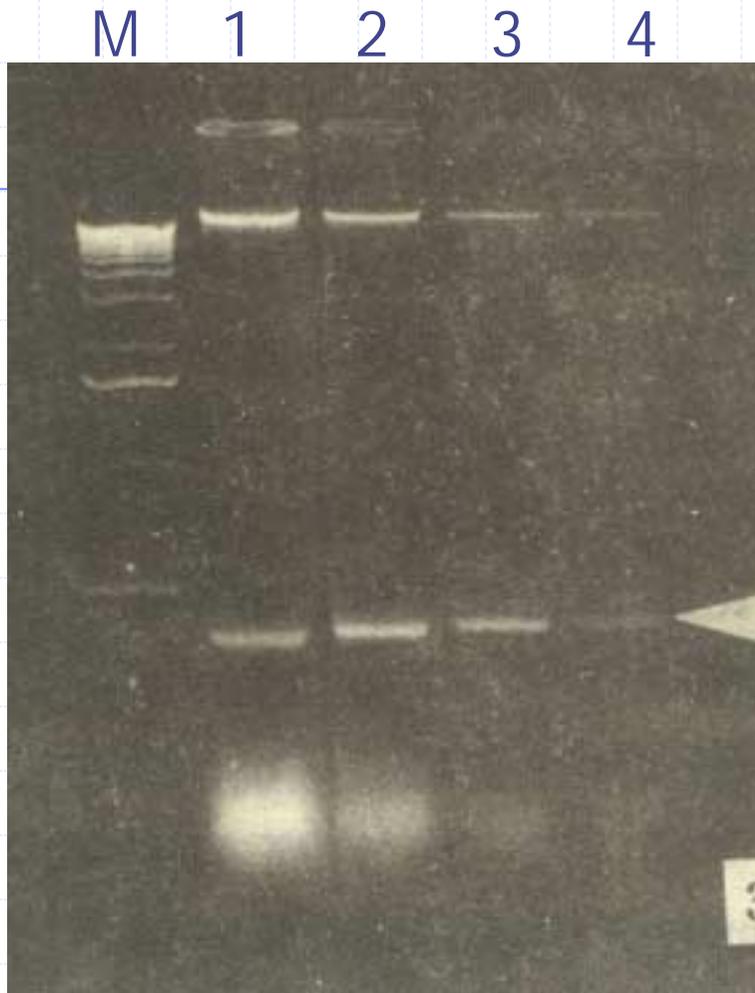
### ◆ 2.1 Detection of tobacco ring spot virus (TRSV)

TRSV is a very important alien quarantine pest in our country.

year	Technique	Sensitivity	Time
1995	RT-PCR	400pg	5h
2000	nitrocellulose IgG-gold	24ng	7h
2002	Gold immunochromatography	1000ng	1-2m
2002	SPA-ELISA kit	150ng	8h



Symptom of PRSV



The result of detection  
TRSV from the infected  
sap by rt-PCR (1995)

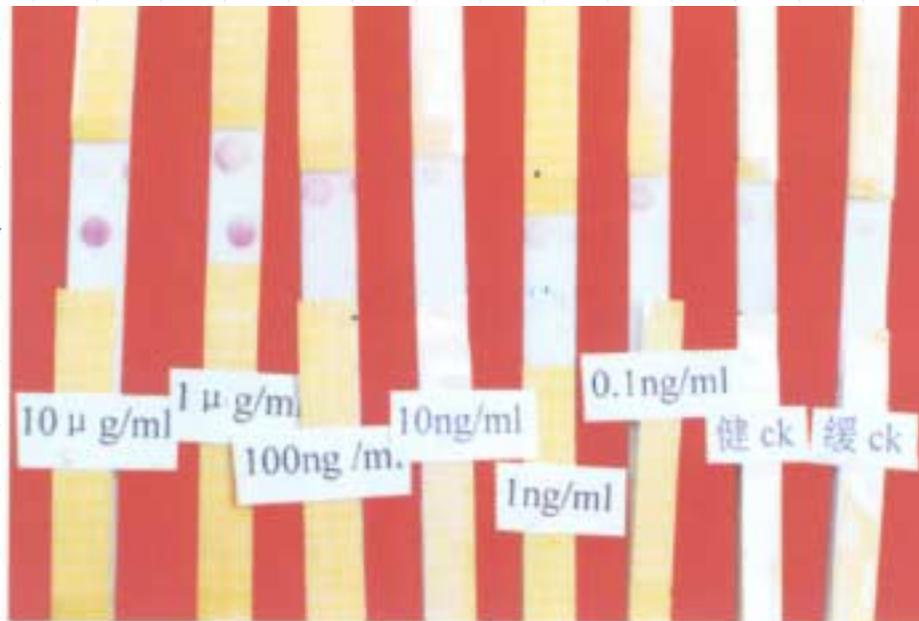
M: marker,

1: 2.4 $\mu$ g,

2: 1.2 $\mu$ g,

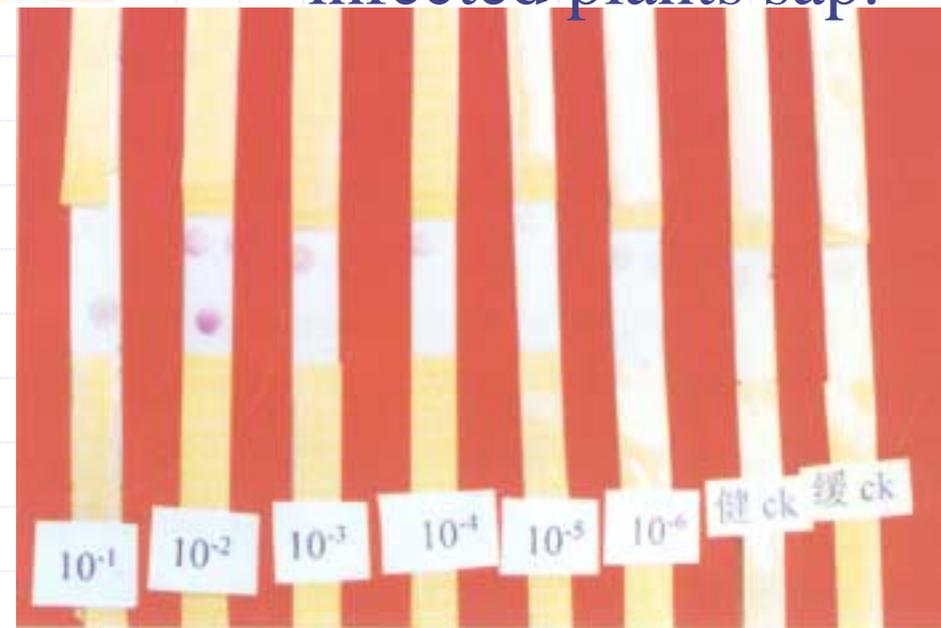
3: 600ng,

4: 300ng)



Result of GICA  
detecting purified  
TRSV(2002).

Result of GICA  
detecting TRSV  
infected plants sap.

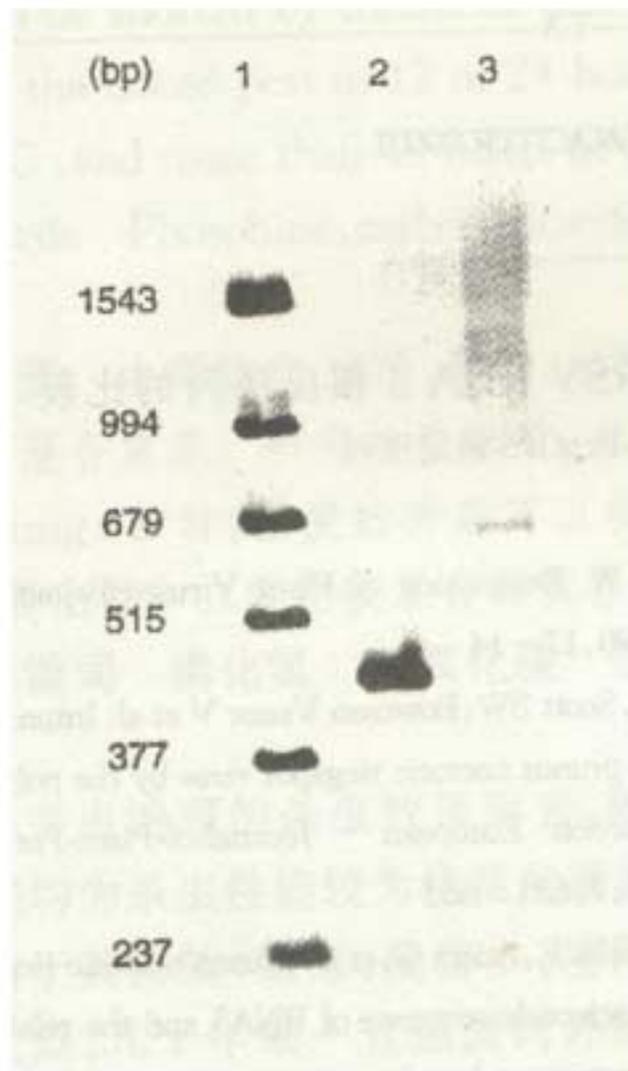


## ◆ 2.2 Detection of Prunus necrotic ringspot virus (PNRSV )

- PNRSV was the padlock import quarantine pest in China. The host range of TRSV is extensive and its symptoms are always changeful.
- For rapid detecting PNRST from plant tissue, a pairs of primers were designed, corresponding the sequence of PNRSV RNA3.
- RT-PCR was gotten on with total RNA of the infected tissue and health sample.
- The target fragment about 450bp was amplified from the infected sample, but not from the health sample.



Symptoms of PNRSV in Rose



The result of detecting  
PNRSV from plant sap  
by rt-PCR.

1: marker,  
2: infected sample,  
3: health sample.

## ◆ 2.3 Study on ELISA of Detecting *Curvularia lunata* in Maize

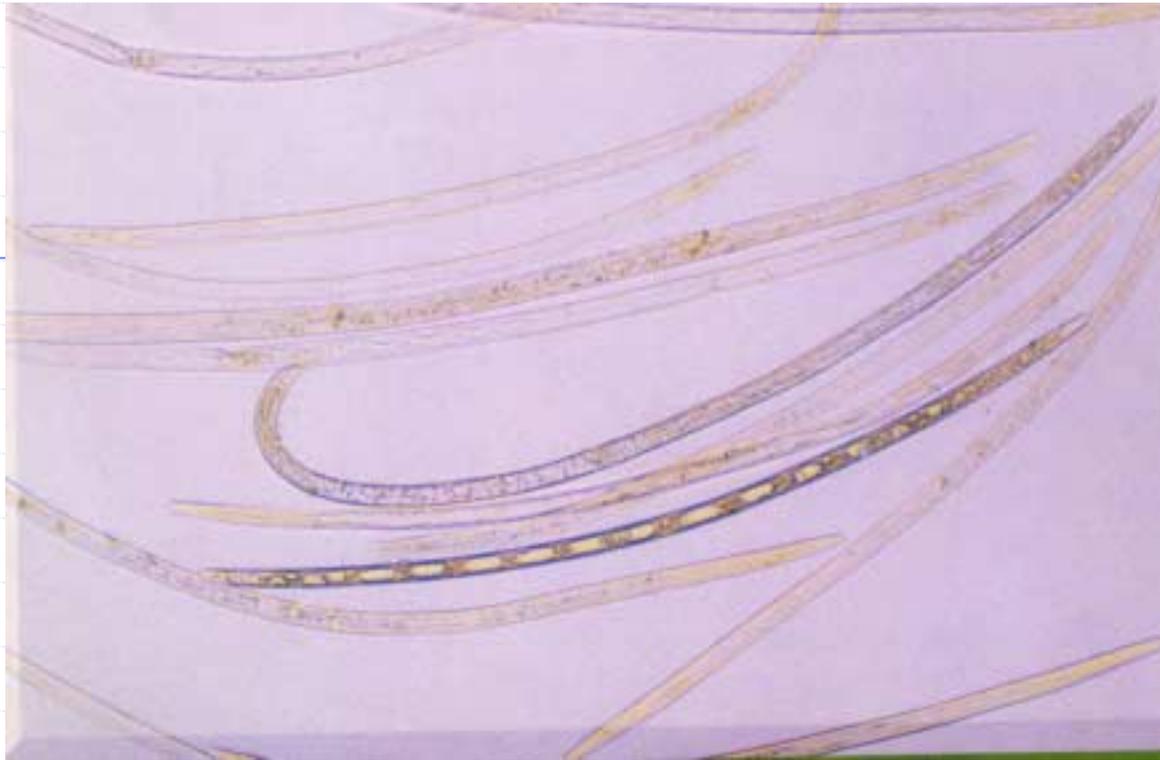
- Maize leaf-spot disease is a very important seed-brought disease which occurred in the north of China these years.
- We design ELISA measures of detecting *Curvularia lunata* in Maize. The antisera of *C. lunata* were absorbed with some similar strains' proteins to eradicate the cross-reacting antibodies.
- It was detected by the ABC-ELISA and indirect ELISA. The sensitivity of ABC-ELISA was 2 to 5 times higher than I-ELISA.



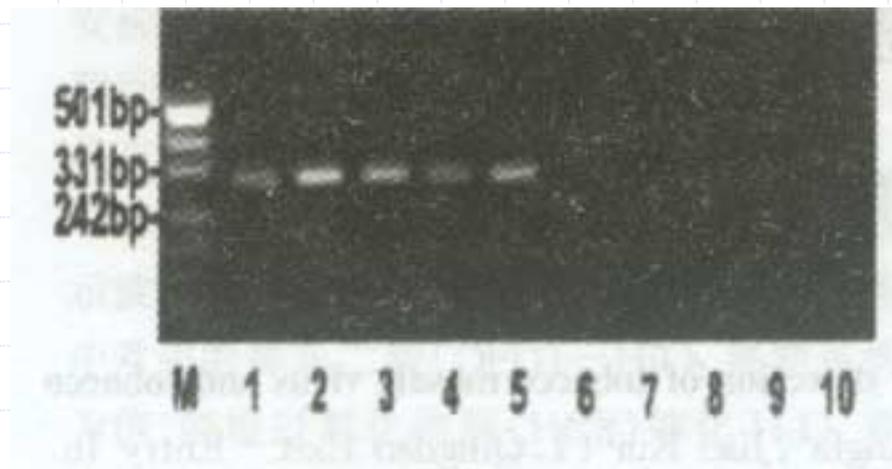
Symptom of Maize leaf-spot disease

## ◆ 2.4 .The speed detection for the pine wilt disease caused by *Bursaphelenchus xylophilus*

- The pine wilt disease is one of the destructive disease in many countries. It is very similar to *B. mucroratus* in morphology.
- To overcome the limitation of the traditional morphological identification of these two organism, We developed a PCR method using two special primers, which depended on the rRNA gene of *B. xylophilus* by changing a base intentionally.
- This method is very practical and stable in quick detection of individual sample for quarantine department.



*B. xylophilus* and  
*B. mucroratus*



Result of detecting  
individual sample by PCR  
M: Marker,  
1-5: *B. xylophilus*,  
6-10: *B. mucroratus*.

### 3. The speed detection for pest of citrus

- ◆ Citrus plays an important role in China. And its production is the third in the world only inferior to Brazil and America.
- ◆ Citrus canker and citrus Huanglong disease are important quarantine disease in the world also in China.
- ◆ According to present plant quarantine laws and rules of China, the plants and trees infected by these disease must be removed and eradicated.
- ◆ Agriculture ministry of China and Chongqing University worked out three speed detection kit for this two diseases. That is useful to surveillance and to establish citrus pest free areas.

### 3.1 The speed detection technique for Citrus canker (*Xanthomonas axonopodis* pv. *citri*)



The immunological  
detect kit for citrus canker (DIA)



It is designed at the base of the traditional method ELISA by using pyroxylin film. We can get the result depending on the colour of the film when the reaction has finished.

# Process

## Preparing samples

0.5g citrus leaf sample in  
distilling (30min)

add 10ul/dot sample to the  
film (15min)



washed three times  
(5min/time)



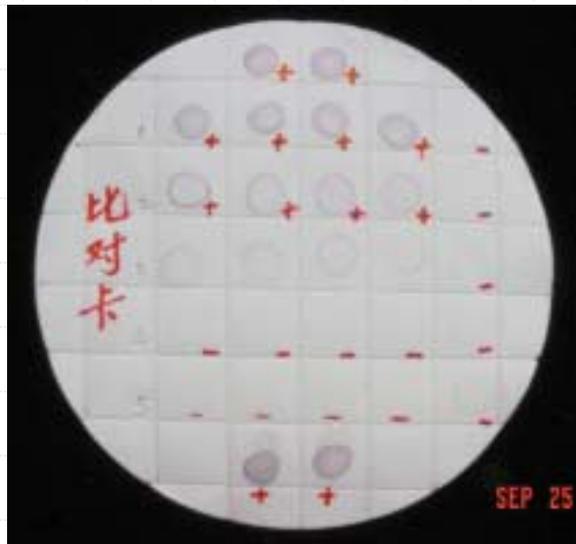
## Reaction and result

washed

incubate in the first antibody diluent  
(1hr)

washed

incubate in the second antibody  
diluent (1hr)



reaction in darkness with enzyme

stop the reaction in water (5min)

Check the result as the card

## Characteristic

- specificity: only citrus canker bacterium reacts with antibody and the colour turns blue.
- sensitivity: the lowest limit to  $1 \times 10^5$  cfu/ml;
- Storage life: half and one year at normal temperature;
- cost: 50yuan/sample.



The PCR detect kit for  
citrus canker

It's primers are designed according to the diversity of DNA fragment. The PCR reaction reagent mix with biology stable reagent and freezing-dry. The liquid sample was added to the reaction reagent before PCR. We can confirm the result by comparing with positive standard.

## process



- ◆ 7~8 pieces of clipped citrus sample with  $7 \times 15$ mm are added to 1ml distilling liquid (15min)
- ◆ add 5 $\mu$ l/tube sample for detection (15min) to solid PCR tube



Add 20ul recovering liquid to every tube to PCR (1.5hr)



The result of PCR production.  
The target fragment is about 400bp.

## Characteristic

- ◆ Easy to prepare sample : special buffer for DNA preparation. It can be finished in 20~30min.
- ◆ Detection diversity: the PCR primer pair XAC01 /XAC02 can effectively detect all strains of citrus canker.
- ◆ Sensitivity: 1.59 pg/ $\mu$ l, 10cfu/25 $\mu$ l
- ◆ Storage life: half of one year in normal temperature.
- ◆ Cost: ¥ 150 /sample

### 3.2 The PCR speed detection technique for citrus Huanglong disease (*Liberibacter asiaticus*)



The PCR detect kit for citrus Huanglong disease



## process



Take 200mg the costa of leaf and break to pieces, then add 0.5ml extracting liquid.

Other steps as before.



The target fragment is about 700bp.

## 4. Application of the speed detection in China

There are many studies on speed detection measures and methods in China. But few are applied in fact, because of:

◆ **shortage of perfect and stable technology:**

Most detecting technique are being improved and studied to meet the need of quarantine pursuit. But there is a distance between study and application.

### ◆ **shortage of outlay:**

The cost of speed detection is higher than others, some perfect measures can't become production. They are only applied in scientific research groups other than local user.

### ◆ **small lots and quantities of detecting:**

The consumers of some production (e.g. TRSV) are very limited, the produce of corresponding production often decrease gradually even to stop. That brings a lot of inconvenience for quarantine detection.

◆ **the limitation of technology and equipment:**

PCR and ELISA are only applied in scientific research groups, academy and part of provincial plant quarantine labs. Other common labs haven't enough equipments and comparative technique level. If we don't improve those detection methods and make it simple and easy, it is difficult to be used widely.

Thanks!

