Overview of plant quarantine in Shenzhen, China

Zhang GM[1,2,3*], Ling XY[1,3], Feng JJ[1,2,3], Cheng YH[1,2,3], Zheng Y[1,2,3], Li YN[1,2,3], Long H[1,2,3], Li FR[1,2,3], Wang Y[1,2,3], Jiao Y[1,2,3], Xiang CY[1,3], Yu DJ[1,2,3] and Kang L[1,2,3]

[1]Shenzhen Entry-exit Inspection and Quarantine Bureau, Shenzen, 518045 Guangdong P.R., China
[2]State Key Quarantine Laboratory of Legume Pest & Plant Pathogenic Fungi of AQSIQ, Shenzhen, 518045 Guangdong P.R., China
[3]Shenzhen Key Laboratory of Inspection Research & Development of Alien Pests, Shenzhen, 518045 Guangdong P.R., China

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China's entrance to the World Trade Organization and trend towards globalization of its economy and finance has increased international trade, especially in agriculture and forestry products. Plant inspection and quarantine has correspondingly grown and developed in importance. In this paper, plant quarantine in Shenzhen, including its team, detection platforms, inspection protocols, scientific research, achievements and collaborations will be introduced. The Plant Inspection and Quarantine Laboratory in Shenzhen City is now one of the largest laboratories in this field in China. Two national and regional key laboratories have been built, ranking top both in inspection capacity and routine inspection. In 2010, more than 70,000 imported products were inspected, and more than 30,000 pest-infected products were intercepted. By carrying out scientific research and cooperating domestically and abroad, the laboratory is leading the way in the development of standard methods for standard pest testing using morphology, molecular biology and immunology. The Shenzen Inspection and Quarantine Bureau therefore facilitates the testing of pests in imported commodities and contributes to the safe and efficient international trade in grains, fruits, seeds, seedlings and other plants and plant products.

Key words – plant inspection and quarantine – detection platform – inspection items

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*Corresponding author: Guiming Zhang – e-mail – zgm2001cn@gmail.com

Introduction

Shenzhen Entry-Exit Inspection and Quarantine Bureau and Plant Inspection and Quarantine Laboratory

Shenzhen City in southern China is situated north of Hong Kong. Owing to China's economic liberalization under the policies of reformist leader Deng Xiaoping, Shenzhen became China's most successful "Special Economic Zone". Shenzhen Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, also called SZCIQ (Fig.1), is directly under the General Administration of Quality Supervision, Inspection and Quarantine, of the People's Republic of China (AQSIQ) and is one of the largest authorities charged with inspection and quarantine of imported and exported products among 35 authorities in China. At present, SZCIQ is the only authority that is authorized to take the responsibility of health quarantine, animal and plant quarantine, inspection, and identification, certification and supervision of import and export commodities

Fig. 1 – New building of Shenzhen Entry-Exit Inspection and Quarantine Bureau.

in the Shenzhen area. SZCIQ has more than 3,000 well-educated employees, including more than 50 with PhD degrees, 407 with Masters degrees and 1,442 with Bachelor degrees. These staff work in its 20 departments or divisions, two sub-bureaus and 11 branch bureaus, situated at its 11 different ports and eight direct affiliates.

The Animal & Plant Inspection and Quarantine Technology Center (APIQTC) is one of the five technical centres that is under the supervision of SZCIQ. The Plant Inspection & Quarantine Laboratory(PIQL) was incorporated in APIQTC in 2000, was founded in 1964 and is called the Laboratory of Shenzhen Plant Quarantine Institution. It is the only agency responsible for the inspection and quarantine of imported and exported plants and their products in Shenzhen Port. PIQL is mainly in charge of inspection of fungi, bacteria, viruses, nematodes, insects, weeds and other pests in imported and exported plants and their products, quality inspection of agricultural products as well as detecting of genetically modified products. Two key laboratories, namely the “State Key Quarantine Laboratory of Legume Pest & Plant Pathogenic Fungi of AQSIQ” and “Shenzhen Key Laboratory of Inspection Research & Development of Alien Pests” have been developed in the plant inspection and quarantine laboratory. The laboratory was accredited by the China National Accreditation Board for Laboratory (CNAL) according to ISO/IEC 17025 in 2003 and passed the laboratory assessment of certification and accreditation chaired by China National Accreditation Services for Conformity Assessment (CNAS) in 2009. PIQL has the capability to carry out 1123 inspection items, among which 154 inspection items were certified or accredited by CNAS. PIQL employees include 26 technical employees, including six senior researchers, nine senior agronomists, seven agronomists, two assistant agronomists and two technicians. Eight employees have a PhD, nine a Masters degree and all are employed with the expertise covering nearly all professional disciplines for plant inspection and quarantine, including fungi, viruses, bacteria, nematodes, insects, weeds, GMO detection and quality inspection.

The Detection Platforms

The laboratory is well equipped with more than 50 million RMB in detection equipment which includes technically advanced and expensive instruments such as a CLSM (Confocal Laser Scan Microscope), EM (electric microscope), ZEISS advance microscopes,

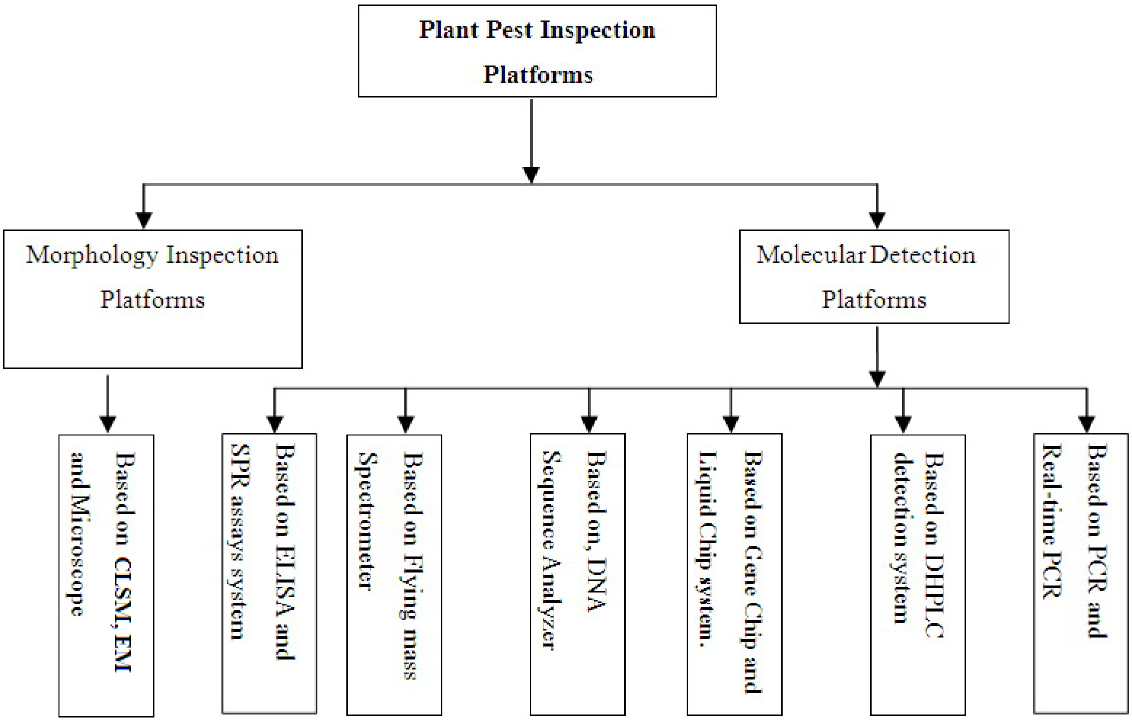

Fig. 2 – Platforms for Plant Pest Inspection

Microarray spotter, Microarray scanner, ABI 3,100 genetic analyzer, ABI 7,900 quantitative PCR, Liquid gene-chip detection system, DHPLC (denature high pressure spectrometer), Flight mass spectrometer, Automatic workstation for nucleic acid abstraction and ProteON-XPR36 protein interaction assays system (SPR). The items have been equipped to meet the needs of the different professional disciplines. Platforms for advanced pest inspection and quarantine both in morphological identification and molecular detection, including the detection platform of PCR, real-time PCR and multiplex PCR, the detection platform of DNA chip, the detection platform of ELISA, the detection platform of DHPLC, the detection platform of Flight mass spectrometer and the advanced morphological detection of microscopy, were established based on modern and conventional equipment (Fig. 2). With these platforms, more than 70 standard detection methods for plant pests have been developed, including those based on real-time fluorescence quantitative detection, gene chip high-throughput & DNA detection and morphology inspection (Fig. 3).

Inspection Item

The testing scope covers eight fields, as showing as follow Fig. 4.

Testing for various pests

Fungi – The laboratory mainly focuses on identification of the pathogenic fungi from imported and exported grains, fruits, tobacco leaves, and plant propagation materials. The staff include two research scientists, one senior agronomist, and two assistant agronomists. The laboratory is also devoted to develop standards for testing methods using morphology and molecular biology. More than ten standard protocols including methods for detection and identification of *Tilletia indica*, *T. walker and Phytophthora sojae*, criteria for the identification and detection of fungi, and operation procedures for the import and export wheat flour Quarantine, have been developed. Detection platforms for morphology, pathogenic activity and molecular biology have been developed, and plant pathogenic fungi such as *Tilletia indica* (Fig. 5), *Diaporthe phaseolorum* var. *meridionalis* (Fig. 6), *Tilletia contraversa* and *Peronospora hyoscyami* f.sp. *tabacina* have been identified from imported plants on many occasions. Over the past 10 years, molecular phylogenetic analysis of *T. indica* and its related species were investigated using ITS nrDNA sequence data. For reliable, sensitive and rapid detection methods for quarantine inspection 'detection of *T. indica* by 5'-nuclease assays using TaqMan MGB probe, and duplex-

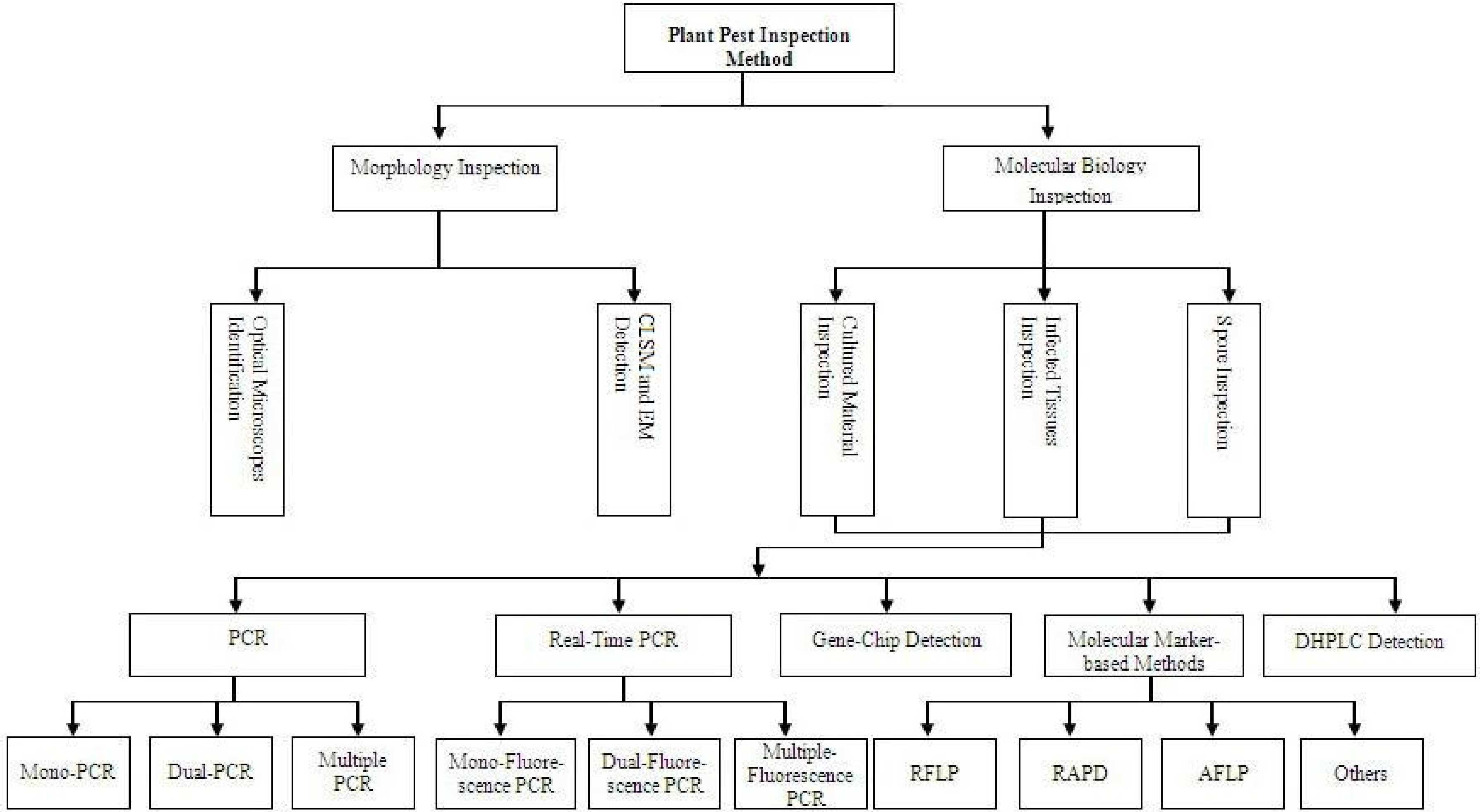

Fig. 3 – Inspection Methods for Plant Pests.

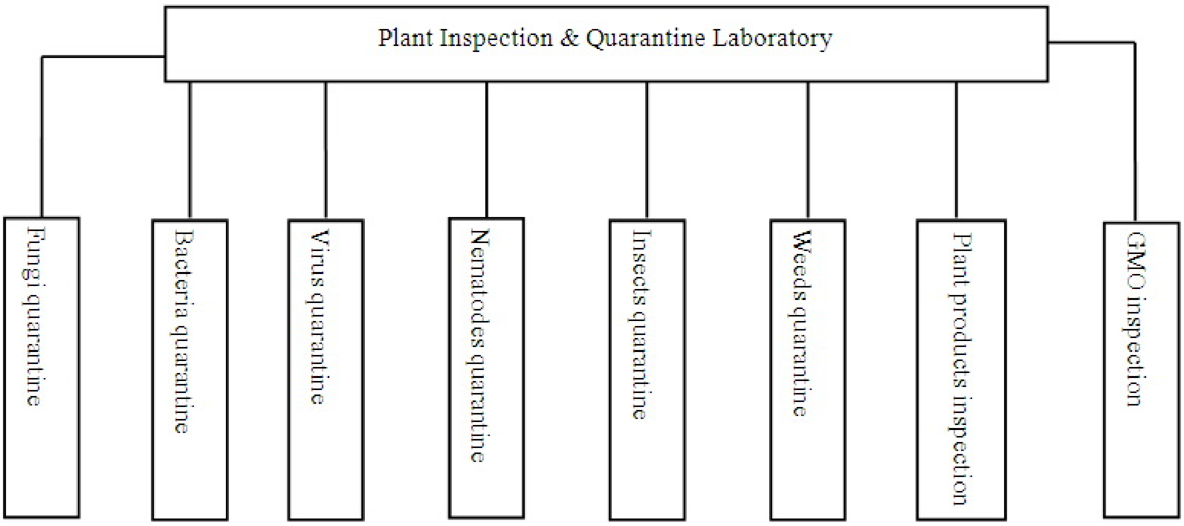

Fig. 4 – Testing scope of the Plant Inspection & Quarantine Laboratory.

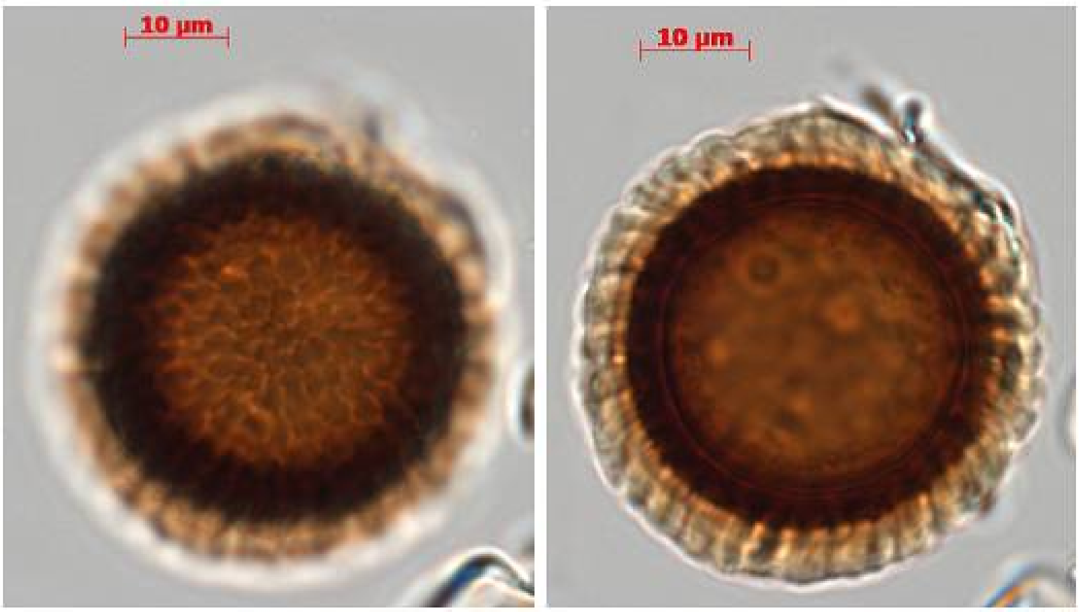

Fig. 5 – Spores of *Tilletia indica* under light microscopy.

real time fluorescent PCR, and DNA chip were developed successfully (Zhang et al. 2005, 2006). A method of braking single spores and using molecular detection has also been developed (Cheng et al. 2001). Pest risk analysis of *T. indica* in China using the Humid Thermal Index and GeoPhytopathology models have also been developed (Zhou & Zhang 2010). This team has also described for the first time a robust assay for reliable identification of *Verticillium* spp. using protein fingerprinting data obtained by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF-MS) (Tao et al. 2009). A monograph entitled "Establishment of plant pathogenic fungi test platform-detection" has also been published (Zhang et al. 2005).

Bacteria – Attention to bacteria of quarantine significance from imported seeds, seedlings, and fruits using the Biolog MicroStation system, ELISA, PCR and other methods is the main focus. This work is carried out by one agronomist whose research focuses on characterization and detection of 57 bacteria on the Chinese government quarantine list. The agronomist has developed a multilocus typing sequence assay for characterizing and tracking *Acidovorax avenae* subsp. *citrulli* (Fig. 7) in

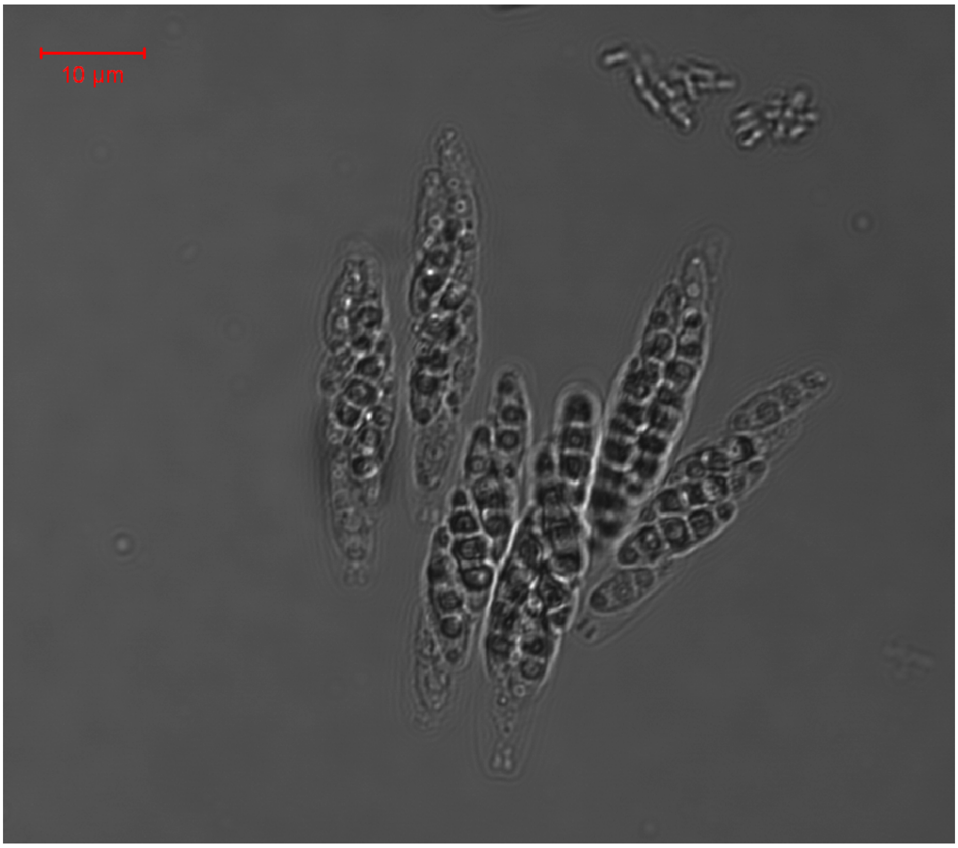

Fig. 6 – *Diaporthe phaseolorum* var. *meridionalis* under light microscopy.

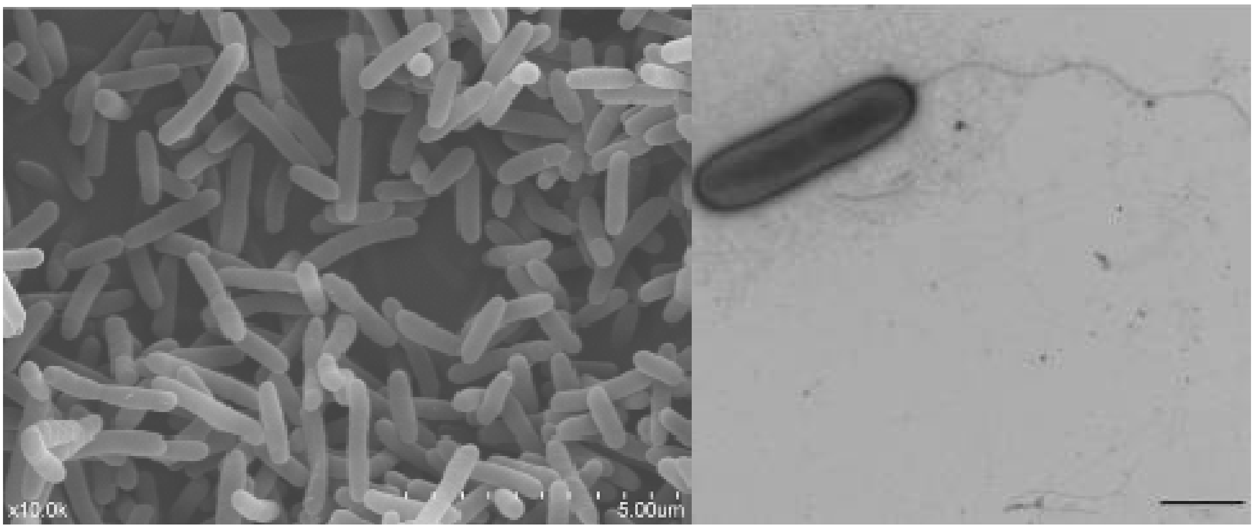

Fig. 7 – Scanning electronic microscopy and transmission electron microscopy of *Acidovorax avenae* sub sp. *citrulli*.

China. This is the causal agent of bacterial fruit blotch of watermelon and other cucurbits (Feng et al. 2009). A real-time BIO-PCR assay for detecting *A. avenae* subsp. *citrulli* in watermelon and melon seeds has also been developed (Zhao et al. 2009). The agronomist has spent 14 months at USDA, Agricultural Research Service, Foreign Disease Weed Science Research Unit, Fort Detrick, MD. At present, he is devoting his time to developing identification methods for microorganisms using matrix assisted laser desorption/ionization time-of-flight mass spectrometry.

Viruses – One senior agronomist is in charge of detecting plant viruses from the entry-exit propagating materials, such as in seeds and seedling using serology, PCR, and semeiology. He also grows seedlings in order to observe any disease symptoms in the quarantine greenhouse. His research focuses on RT-real time PCR or multiplex RT-real time PCR for detection of plant viruses (Yang et al. 2007, Zheng et al. 2010), and Tobacco Ringspot Virus (TRSV), Lily Symptomless Virus (LSV), while other viruses such as Arabis Mosaic (AMV) and Bean Pod Mottle Virus (BPMV) have been detected frequently from imported bulbs and flowers.

Nematodes – The group includes one research scientist, one senior agronomist, and one agronomist. The mission is quarantine of plant parasitic nematodes. Currently the group focuses on taxonomy, phylogeny, morphology, and biology of nematodes from natural and agricultural ecosystems, including plant-parasitic and virus-vector nematodes. We integrate traditional morphology with molecular approaches in our research, establishing PCR methods to detect *Bursaphelenchus xylophilus* and real-time PCR to detect *Radopholus similes*. The group is trying to establish Time of Flight Mass Spectrometry for detection of nematodes. *B. xylophilus*, *R. similes Pratylenchus penetrans* and other nematodes were intercepted from plants and plant products imported from the United States, Europe, Japan and other countries. We have intercepted and stored number of *B. xylophilus* and *R. similes* groups. We have been involved in drafting the pest list for Hong Kong, trained internal and external staff, cooperated with domestic and overseas universities and research institutions, and chaired over Proficiency Testing of *Meloidogyne* spp., developing detection standards and have published numerous of articles in core journals in China (Long et al. 2008, 2010).

Insects – The staff include two research scientists, one senior agronomist, two agronomists, and one technician, who are responsible for detecting harmful insects from fresh fruits, wood, beans, plant propagation materials, vegetables and other plant products. More than 50,000 batches of plant products are inspected each year and about 650 species of fruit insect, forest insect, storage insect and vegetable insect were intercepted and have been identified. These include *Callospbruchus maculaes*, *Ceratitis capitata* (Fig. 8), *Hylurgus ligniperda*, *Quadrastichus erythrinae* and *Solenopsis invicta* and *Planococcus lilacinus*, The team is interested in morphological and molecular identification of insect pest and quarantine treatment techniques. More than ten research projects had been conducted funded by the Ministry of Science and Technology of PRC, AQSIQ, Shenzhen Municipal Government and Shenzhen-CIQ in the previous decade. Conventional PCR, real-time PCR, microarray and reverse Dot Blot (RDB) techniques have been applied for rapid detection for Tephritidae fruits flies, Bostrychidae and Platypodidae beetles and Pseudococcidae scales intercepted from the international plant products (Yu et al. 2005, 2007). We have published eight standard protocols such as Rules for Quarantine of *Solenospis invicta* (GB/T 23634-2009).

Weeds – This team involves one research scientist and one agronomist who are responsible for detecting harmful weeds from imported or exported soybeans, wheat, rice, bean, sesame, rapeseed, vegetable seeds, tobacco and other seeds using conventional methods, such as macrography and microscopy. Since 2001, the team has intercepted hundreds of harmful weeds or relative seeds from imported plant products in Shenzhen Port, including *Sorghum halepense*, *Crotalaria separase*, *Datura stramonium*, *Lolium temulentum*, *Cuscuta* sp., *Orobanche* sp. and others. Furthermore, this team takes charge of monitoring exotic harmful weeds in Shenzhen on behalf of the AQSIQ.

Plant Products Quality – Plant product quality inspection is responsible for quality inspection of the import and export of agricultural products using sensory inspection methods (appearance, colour, odour, taste) and physical and chemical testing. This team involves two agronomists and one technician, and KJELTEC 2300 automatic Kjeldahl apparatus, SOXTEC 2050 automatic soxhlet extraction apparatus, PERTEN1900 falling number instrument, TX7 Carter removing impurity machine, Infratec 1241 near infrared quality analyzer, and other advanced instruments. Inspected products include wheat, barley, soybean, corn and other grains imported from the United States, Australia, Canada, Brazil, Argentina, France and other countries. The main testing scope includes impurity, density, dockage, purity, damage kernel, thermal damage kernel, weight,

Fig. 8 – *Ceratitis capitata*.

germination, falling number, moisture content, protein content and crude fat content. Totally 10,915,000 tons of crude imports were inspected since 2002, and 2,008,000 tons of goods below standard were detected. Unqualified items include impurities, density, thermal damage and crude protein content of the grain.

Detection of genetically modified plants: The Plant Inspection and Quarantine Laboratory is one of the 18 detecting laboratories for genetically modified plants that were accredited for the first time in China. The laboratory focuses on GMO detection in soybean, corn, rapeseed, rice and others with detection platforms such as qualitative PCR, quantitative PCR, solid chips, liquid chips, and other methods. The GMO laboratory has the ability to detect GMO in corn and rice or lines and rice products. The GMO laboratory intercepted unauthorized GM corn in 2010 for the first time in China.

Inspection and Interception

Testing has increased six fold in the past 10 years, increasing about 20% yearly, from 11,243 batches in 2001 to 69,547 batches in 2010 (Fig. 9). Detection rates have increased from 6659 batches in 2001 to 41,906 batches in 2010. In 2010 alone, in 41,906 samples; totally 948 species of pest were intercepted, including 631 species of insect, 243 species of weeds, 46 species of fungi, 17 species of bacteria, 9 species of nematodes and two species of virus. Sixty-nine species were identified to be the quarantine pests, including 43 species of insects, 17 species of weeds, four species of nematodes, one species of virus and four species of fungi subject to quarantine, with 25.8% more records than in 2009 (Table 1).

Research and Collaboration

The Plant Inspection & Quarantine Laboratory has concentrated on scientific research to develop new methods to solve the problems of rapid and high-throughout testing. In the past five years, the laboratory has completed more than 40 scientific research programs. The laboratory has also drafted or published more than 30 National or SN standard protocols for quarantine and inspection, more than 100 research publications and three books, and applied for seven invention patents with five authorized. This laboratory has continued to strengthen domestic and international communications and cooperation in recent years. The laboratory has built communication and cooperation with the Mushroom Research Foundation in Thailand and with colleagues in, Canada, Hong Kong, Philippines and the USA. In the technical field, the laboratory has cooperated with the Chinese Academy of Science, Chinese Academy of Inspection and Quarantine, National Institute of Metrology, China and South China Agricultural University, Hunan Agricultural University, Guizhou University and Sun Yat-Sen University.

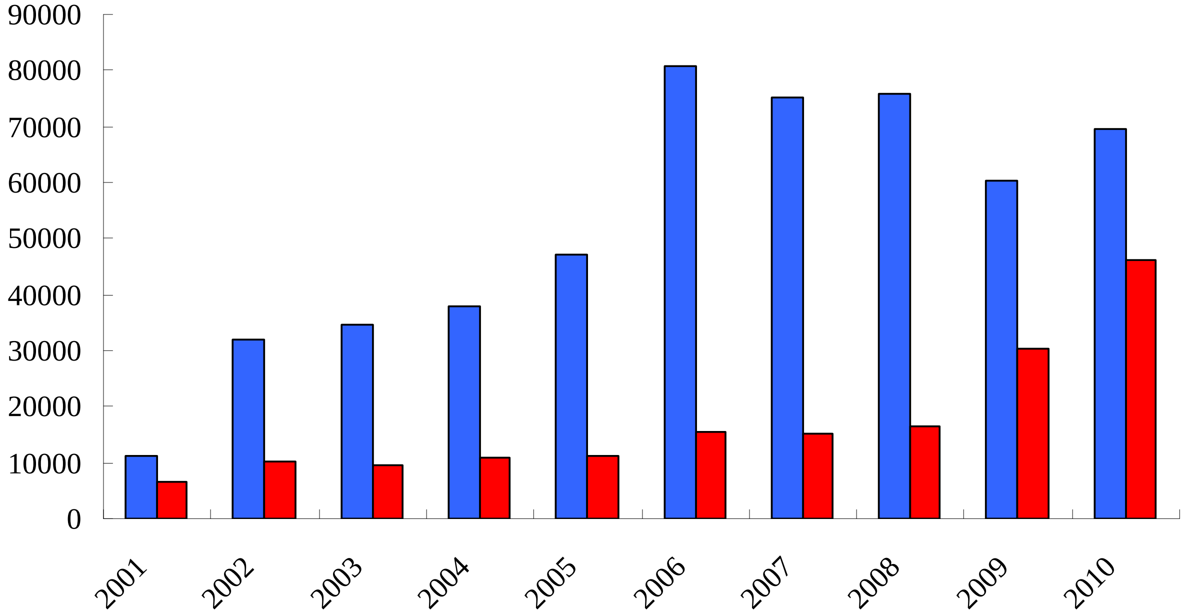

Fig. 9 – Number of batches tested by the Plant Inspection & Quarantine Laboratory between 2001–2010.

Table 1 Interception of quarantine pests in 2010.

Number	The scientific name of quarantine pests	Scope	Countries and regions
1	*Acanthoscelides obtectus*	Quarantine insects	Uncertain*
2	*Achatina fulica*	Quarantine mollusca	Uncertain
3	*Agrilus* spp. (non-Chinese)	Quarantine insects	Canada
4	*Bactrocera correctus*	Quarantine insects	Thailand
5	*Bactrocera cucurbitae*	Quarantine insects	Uncertain
6	*Bactrocera dorsalis*	Quarantine insects	Algeria, Cambodia, Malaysia, South Africa, Thailand, India, Indonesia, Vietnam, Taiwan
7	*Bactrocera latifrons*	Quarantine insects	Malaysia, Thailand, India
8	*Bactrocera philippinensis*	Quarantine insects	Philippines
9	*Bactrocera rubiginus*	Quarantine insects	Uncertain
10	*Bactrocera scutellata*	Quarantine insects	Uncertain
11	*Bactrocera* sp.	Quarantine insects	Thailand
12	*Bactrocera tau*	Quarantine insects	Uncertain
13	*Callosobruchus analis*	Quarantine insects	Uncertain
14	*Callosobruchus maculatus*	Quarantine insects	Australia, Brazil, Philippines, Korea, USA, Myanmar, Japan, Saudi Arabia, Jamaica, India, Indonesia, Vietnam
15	*Ceratitis capitata*	Quarantine insects	Mauritius
16	*Coptotermes sjostedi*	Quarantine insects	Benin
17	*Coptotermes* spp. (non-Chinses)	Quarantine insects	Brazil, Guyana, Malaysia, Thailand, Vietnam
18	*Crossotarsus externedentatus*	Quarantine insects	Thailand, Vietnam
19	*Crossotarsus* spp. (non-Chinese)	Quarantine insects	Myanmar, Canada
20	*Dacus ciliatus*	Quarantine insects	Uncertain
21	*Dendroctonus valens*	Quarantine insects	Canada
22	*Heterobostrychus aequalis*	Quarantine insects	Malaysia, Thailand, Vietnam
23	*Hylurgus ligniperda*	Quarantine insects	Australia, New Zealand, Chile
24	*Hypothenemus hampei*	Quarantine insects	Ethiopia, Brazil, Uganda, Indonesia, Vietnam
25	*Ips grandicollis*	Quarantine insects	Australia

Table 1 (Continued) Interception of quarantine pests in 2010.

Number	The scientific name of quarantine pests	Scope	Countries and regions
26	*Planococcus lilacius*	Quarantine insects	Malaysia, Thailand
27	*Planococcus minor*	Quarantine insects	Thailand, Vietnam
28	*Platypus cupulatullus*	Quarantine insects	Malaysia, USA, Thailand, Indonesia, Vietnam, Taiwan
29	*Platypus cupulatus*	Quarantine insects	Malaysia, USA, Thailand, Vietnam
30	*Platypus flavicornis*	Quarantine insects	USA
31	*Platypus cylindrus*	Quarantine insects	Malaysia
32	*Platypus parallelus*	Quarantine insects	Australia, Paraguay, Panama, Brazil, Bulgaria, Benin, Belgium, Bolivia, Germany, France, Philippines, Costa Rica, Guyana, Canada, Gabonese, Cambodia, Madagascar, Malaysia, USA, Myanmar, Mexico, South Africa, Slovakia, Suriname, Thailand, Singapore, Indonesia, Vietnam, Chile, aiwan
33	*Platypus solidus*	Quarantine insects	Malaysia, Thailand, Vietnam
34	*Platypus* spp. (non-Chinese)	Quarantine insects	Canada, Romania, Malaysia, Thailand, Taiwan
35	*Prostephanus truncatus*	Quarantine insects	USA
36	*Sinoxylon* spp. (non-Chinese)	Quarantine insects	Malaysia, Thailand
37	*Sinoxylon conigerum*	Quarantine insects	Germany, Ghana, Laos, Malaysia, Mexico, Thailand, Singapore, India, Indonesia, Vietnam
38	*Sinoxylon crassum*	Quarantine insects	Canada, Malaysia, Thailand
39	*Solenopsis invicta*	Quarantine insects	Australia, USA, Suriname, Italy
40	*Sternochetus maniferae*	Quarantine insects	Malaysia, South Africa, Uganda
41	*Sternochetus olivieri*	Quarantine insects	Uncertain
42	*Trogoderma anthrenoides*	Quarantine insects	Uncertain
43	*Xyleborus* spp.	Quarantine insects	Belgium, Germany, France, Canada, Malaysia, USA, Slovakia, Thailand, India, Indonesia, Vietnam, Taiwan
44	*Ambrosia artemisiifolia*	Quarantine weeds	Brazil, Canada, USA
45	*Ambrosia trifida*	Quarantine weeds	Canada, USA
46	*Bromus rigidus*	Quarantine weeds	Australia, Italy, UK
47	*Cenchrus echinatus*	Quarantine weeds	Brazil
48	*Cenchrus longispinus*	Quarantine weeds	Argentina
49	*Cenchrus tribuloides*	Quarantine weeds	Argentina
50	*Emex australis*	Quarantine weeds	Australia
51	*Euphorbia dentata*	Quarantine weeds	Brazil
52	*Solanum rostratum*	Quarantine weeds	USA
53	*Sorghum almum*	Quarantine weeds	Argentina
54	*Sorghum halepense*	Quarantine weeds	Argentina, Australia, Brazil, USA, Uruguay
55	*Xanthium strumarium*	Quarantine weeds	USA, Argentina, Australia, Canada, Uruguay
56	*Xanthiun strumarium* var. *canadensis*	Quarantine weeds	USA
57	*Emex spinosa*	Quarantine weeds	Australia
58	*Cuscuta* spp.	Quarantine weeds	Bangladesh
59	*Lactuca pulchella*	Quarantine weeds	Brazil
60	*Avena ludoviciana*	Quarantine weeds	Australia, Canada, USA, UK
61	*Diaporthe phaseolorum* var. *caulivoar*	Quarantine fungi	Argentina, USA

Table 1 (Continued) Interception of quarantine pests in 2010.

Number	The scientific name of quarantine pests	Scope	Countries and regions
62	*Diaporthe phaseolorum* var. *meridionalis*	Quarantine fungi	USA
63	*Leptosphaeria maculans*	Quarantine fungi	Canada
64	*Monilinia fructicola*	Quarantine fungi	USA
65	*Bursaphelenchus xylophilus*	Quarantine nematodes	USA
66	*Meloidogyne* sp.	Quarantine nematodes	Uncertain
67	*Pratylenchus* sp.	Quarantine nematodes	Uncertain
68	*Longidorus* sp.	Quarantine nematodes	Uncertain
69	Bean pod mottle virus	Quarantine virus	Uncertain

* Uncertain: Intercepted from passengers

Plant inspection and quarantine has played a critical role in reducing the risk of international trade for over 30 years and helps to maintain China's agriculture and forestry free from some of the world's most severe pests and diseases. In the past ten years alone, with other laboratories in China, we have made contributions for safe international trade in agriculture and forestry products. We understand that we will face further challenges as China is developing and participate in more world trade. As a plant inspection and quarantine laboratory that is situated in one of the largest frontier cities, we expect to take on even more responsibility. We look forward to this challenge and welcome exchange, visits and cooperation with domestic and foreign experts.

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