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The status of quarantine regulation on plant virus and its challenges

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Research

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ABSTRACT

Quarantine regulation has been developed all over the world to minimize impact of pests and diseases via containing the harmful organisms at the center of origin. In this paper, we analysed the detection records of plant viruses in order to gain an insight into the current status of quarantine regulation in China. Meanwhile challenges facing plant virus detection were also listed. The ability to provide a fast, inexpensive and reliable diagnostic for any given viral infection remains a key parameter in efforts to prevent and control these ubiquitous pathogens.

Keywords: quarantine regulation, plant virus, detection methods, challenge, biosecurity

INTRODUCTION

Plant bio-security is vital with its aim to protect the distinct way of life from certain plant pests that can harm the economy through loss of crop production and market access, to ensure food security, and to protect the environment [1]. Quarantine has been used for centuries in an effort to prevent the introduction, transmission, and spread of communicable diseases [2]. While plant quarantine is a bio-security measure designed to reduce the introduction and spread of economically important pests of plants or plant products that are not yet present in one area or that are present but do not widely occur and are under official control [3-4].

The term plant pests mostly refers but not limited to insects, viruses, bacteria, fungi, and nematodes. In this study, we focus on plant viruses, especially those that can cause great ecological and economic loss. Viruses present a major threat to humans, agriculture and the ecosystem in general with new viruses emerging every year, such as the new coronvirus in Hubei China recently. Yet plant quarantine is designed to make sure plant material be thoroughly tested and found free of pathogens of concern before importation. Illegal introductions can cause major mayhem, as was, the case of introduction of *cucumber green mottle mosaic virus* into China [5].

In this paper, we analysed the detection records of plant viruses in order to find out:

1) What was the most challenging invasion in the past two years?
2) What should quarantine regulation focus on in the future?
3) How to improve inspection efficiency?

Overall detection in past two years

According to the data provided by China's General Administration of Customs, 2355 batches of plant virus associated productions have been intercepted by China Customs nationwide, including 522 batches related with quarantine viruses, in recent two years (2018.1.1~2020.1.1). Among those, 96% (2253 batches) introduction of plant virus took place via cargo importation where 499 cases of quarantine viruses were included. Travel inspection has intercepted 55 batches of plant virus associated goods carried by travelers, including 12 cases of quarantine viruses (table 1). Reasons for this phenomenon can be:

1) The huge amount and diversity of imported cargoes lead to higher rate of introduction of harmful organisms.
2) Quarantine regulation puts the focus mainly on cargo inspection with enforced risk analysis and later on sampling percent.

Table 1. analysis of plant viruses detected in multiple ways

category	Total No. (percent)	Quarantine viruses (percent)
cargo	2253 (95.67%)	499 (95.59%)
travel	55 (2.34%)	12 (2.30%)
post	42 (1.78%)	8 (1.53%)
container	3 (0.13%)	2 (0.38%)
transportation	2 (0.08%)	1 (0.19%)
sum	2355	522

Cargo inspection

In the past two years, plant viruses were detected as many as 2,253 times in cargo inspection nationwide, of which 80% (1813 batches) were related to soybeans or its products followed by seedlings (16%) (Fig.1). Among them, *soybean mosaic virus* was the most frequent virus (70%) followed by *bean pod mottle virus* (11%). *Soybean mosaic virus* is a common virus infecting soybeans and is widely spread globally. Detection of this virus is mostly associated with imported soybean germplasm from Brazil. As with *bean pod mottle virus,* a quarantine harmful organism, was mainly captured on soybean seeds or related materials and 84% were introduced from U.S. Thus, soybean products have the highest virus interception rate among the imported cargoes. A total of 499 batches of quarantine virus related goods were intercepted, among which the highest frequency was *bean pod mottle virus* (51%, 254 batches), followed by *prunus necrotic ringspot virus* (17%)(Table.2). *Prunus necrotic ringspot virus* and *Arabis mosaic virus* of which the host range was quite wide were mainly derived from the imported seedlings. Information above provides quarantine regulation with the work points to pay attention to in the near future from certain level.

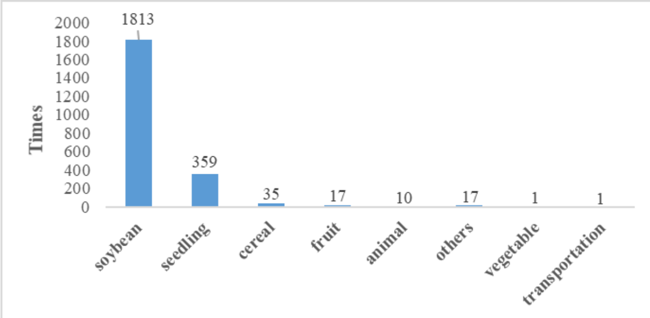

Fig.1 detection analysis of plant viruses in cargo inspection

Table 2. major quarantine viruses detected in cargo inspection in recent two years

Latin name	times	Percent (%)
Bean pod mottle virus	254	50.90
Prunus necrotic ringspot virus	83	16.63
Arabis mosaic virus	46	9.22
Tomato spotted wilt virus	31	6.21
Wheat streak mosaic virus	29	5.81
Cucumber green mottle mosaic virus	18	3.61
Strawberry latent ringspot virus	16	3.21
Tobacco ringspot virus	11	2.20

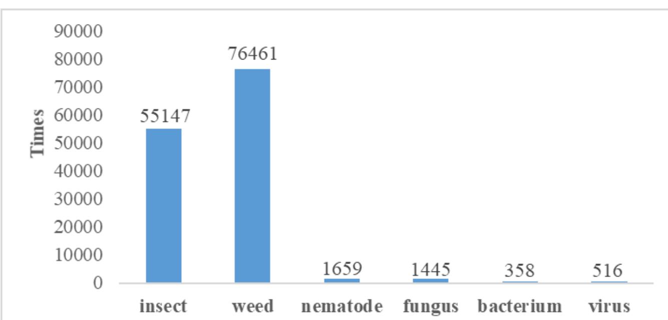

Fig.2 harmful organisms intercepted in past two years

The challenges

The interception rate of plant viruses is lower than that of insects, weeds, or nematodes (Fig.2). Reasons for this phenomenon can be:

1) The biological characters of viruses. The virus particles are small, with diameters ranging from tens to hundreds of nanometers, requiring the aid of an electron microscopy for morphological observation.
2) Fuzzy symptoms. The disease symptom developed by virus infection is limited, indistinguishable, and sometimes similar to that of insect or fungal infection. Certain inspection requires a period of growth before some pests, especially viruses and vascular-limited bacteria reach detection limits or symptoms can have an obvious development [6].
3) Low efficiency of existing methods. The virus titer of certain samples does not reach the detection limit of the testing method, resulting in false negative.
4) Biased sampling. Due to the uneven distribution of most viruses in host plants, sampling can be biased, which would also lead to a false negative [7].
5) Inadequate knowledge of unknown viruses and limitations of pest risk analysis have led to the omission of important deadly viruses.

For those problems above, there are solutions.

1) A combination of biological identification and molecular detection technologies improves the specificity and sensitivity.
2) Samples would be cultured for a period of time before testing or continuously monitored to ensure that the virus titer reaches the detection limit.
3) Development and employment of new detecting methods. In plant virus diagnostics, recent developments can be clustered into three categories:
   a. techniques designed to be performed in non-lab locations (e.g., loop-mediated isothermal amplification);
   b. multiplex methods able to detect many viruses in a single test (e.g., Microfluidics);
   c. methods aimed to virus discovery (e.g., next generation sequencing). Despite the rapid development of more elaborate methods for detecting and identifying viruses, few of them get adopted for routine use in labs, though with many claimed advantages [8]. New methods suitable for lab use require operability and repeatability as well.

4) Virus database is in urge of update, especially those unknown and severe viruses of which both biological and genetic information need to be expanded

accordingly. Comprehensive inventories of plant viral diversity are essential for effective quarantine and sanitation efforts.

5) Make a thorough pest risk analysis basing on a complete virus database. Pathogens present a growing threat to food security and ecosystem management. Interactions between plants and their natural enemies are influenced by environmental conditions. Thus information like, global warming and climate change can affect pest ranges and impact, is supposed to be taken into consideration [9].

DISCUSSION

According to the Merriam-Webster Dictionary, quarantine is "a state of enforced isolation or a restraint upon the activities or communication of persons or transportation of goods designed to prevent the spread of disease or pests". Accordingly, quarantine agencies are supposed to minimize the risk of introducing exotic pests and pathogens along with imported goods, with the goal to protect domestic agriculture and native fauna and flora. Yet increasing agricultural production and trade have been playing a main role in disseminating pests. Evidence for a latitudinal bias in host range shifts of pests also indicates a global warming signal [9]. Thus, quarantine can never be absolute, especially when climate change alters the distribution and prevalence of pathogen vectors, and globalization has allowed the extensive flow of goods and people [1].

The safety of regulated plant material exchanges relies heavily on techniques such as PCR, which is only suited to the detection and identification of specific, well characterized pathogens. For pathogens that are unknown, poorly characterized or highly variable, they are useless. As detection technologies become more sensitive and broader in scope [10], there has been a better understanding about how diseases emerge, which is vital for diseases on perennial crops caused via mixed infection of pathogen complexes. The individual components of these complexes can be asymptomatic, but when they function together they can cause dramatic symptoms and economic losses. Over the last decade, an unprecedented number of viruses have been discovered using high throughput sequencing, which resulted in advancement of our knowledge on the diversity of viruses in nature, particularly unraveling the virome of many agricultural crops. However, these new discoveries have often widened the gaps in our understanding of virus biology, the forefront of which is the actual role of a new virus in disease, if any [11]. A combination of sequence independent NGS-based partial viral genome sequencing with sequence-dependent Sanger-based full genome cloning and sequencing is likely to reduce the number of non-intercepted viruses passing through plant quarantine stations, while at the same time alerting authorities to the presence and potential spread of viruses with unknown pathogenic potentials [12]. The major difficulty in these metagenomic methods continues to be the need for better bioinformatics tools to decipher the large data sets [13]. What's more, methods accessible to labs require an industry standard format and high levels of repeatability and reproducibility which make them easy to establish and robust in their use, even for new and inexperienced users [8].

It is of great importance to undertake a policy of manageable risk, which is based on science, and all quarantine measures should be commensurate with the risk related to that specific pest [14]. China now undertakes a pest risk analysis to assess the potential for entry, establishment, and spread of a pest and the biological and economic consequences of such an entry with plants or plant products. With the rapid development in virus detection and biological interpretation, pest risk analysis can be more precise and systematic for a better utility in future quarantine regulation.

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