Biosafety
ISSN: 2167-0331

Ilardi, Biosafety 2014, 3:1
DOI: 10.4172/2167-0331.1000e143

Editorial Open Access

Plant Biosecurity: Diagnosis in Plant Pathology

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Plant biosecurity is a strategic socio-economic issue concerning the protection of natural and managed plant systems from alien and emerging indigenous pests [1]. Globalization has opened up state borders which, along with climate change and landscape modification, have facilitated the dissemination and introduction of alien pests and the evolution of new races, biotypes and strains of indigenous pests, leading to emerging diseases. Pathogens represent one of the most important threats affecting plant ecosystems by compromising their productivity, sustainability and biodiversity. There are many examples of pathogens resulting in high-impact epidemics - both historical and more recent - from the *Phytophthora infestans* causing the well-known Irish potato famine of the 1840s, to the recent outbreaks of *Pseudomonas syringae* pv. *actinidiae*, the etiological agent of kiwifruit bacterial canker, which severely affected the main kiwifruit producing areas worldwide [2].

A plant biosecurity system requires early detection, accurate diagnosis and a rapid response to prevent the establishment and dispersal of pests, thus minimizing the subsequent impact. Many intergovernmental organizations promoting cooperation in plant health have been established within the framework of the International Plant Protection Convention (IPPC, https://www.ippc.int/). In Europe and the Mediterranean basin, the "European and Mediterranean Plant Protection Organization" (EPPO http://www.eppo.int), develops international strategies against the introduction and spread of dangerous pests, promotes pest control methods, and drafts diagnostic protocols.

The diagnosis of fungal, bacterial, phytoplasmal, viral and viroidal pathogens can be carried out using conventional (morphological, biological, biochemical, physiological, nutritional tests), serological and molecular (nucleic acid-based) methods. In the case of typical symptomatology, a presumptive diagnosis can be performed. However, plants can show unspecific symptoms that can be confused with different biotic or abiotic stresses, as in the case of symptoms caused in *Citrus* by '*Candidatus* Liberibacter' spp. [3] which very much resembles a zinc deficiency [4]. In these cases a detection test is necessary to identify the disease's cause. Often multiple assays are needed to confirm or support the diagnosis. To ensure reliable results, a diagnostic method should thus fulfil certain requisites: sensitivity, specificity, reproducibility, repeatability, selectivity [5]. The ease of use, speed and cost should also be taken into account, each characteristic depending on the circumstances of use [6]. In the case of routine diagnosis or surveillance, speed and cost may be more critical than sensitivity or specificity. Conversely, where a pathogen is not known to occur, diagnostic tests are preferable with high levels of specificity, reliability and reproducibility.

Tests and procedures need to be validated to prevent a disconnection between 'developers' (researchers) and 'users' (diagnosticians) of diagnostic assays. Thus inter-laboratory studies (ring-tests) and proficiency tests aimed at evaluating the protocols and the skills of the laboratory, respectively, are also desirable. The use of appropriate validation guidelines may produce universally acceptable results which are useful for phytosanitary action and regulatory decisions [7].

In order to intercept pathogens early it is important to implement monitoring and sampling. The detection of pathogens in symptomless plant material is crucial for prevention. Statistics and modelling are needed to develop correct advanced sampling strategies. This is particularly true in surveillance programmes of global trade products for 'on-site', at 'point of entry', and also 'at-source' inspections.

The bacterium *Xylella fastidiosa* is a typical example of a disease that requires careful monitoring. Pierce's disease of grapevine caused by the subspecies *fastidiosa* is known to have been present in the USA since 1887, but it has never been reported in Europe with the unconfirmed exception in Kosovo [8]. Great concern has always been placed on the threat of *X. fastidiosa* entry in the EPPO region due to the large import of hosts of the bacterium from the USA [9]. Recently, a rapidly spreading decline of aged olive trees has taken place in the major Italian olive producing region, Apulia (Southern Italy). In October 2013 *X. fastidiosa* was associated with these diseased plants and also with oleander and almond [10]. Considering the high number of plant hosts of this bacterium, it is essential to identify the cause of its introduction and the characterization of its subspecies.

Pathogens quickly evolve in order to survive and perpetuate in various environmental conditions. This variability may reflect the fitness in *planta* and/or the virulence of the pathogen. One case of the convergent evolution of different pathogen populations in the same plant genus is the occurrence of at least four genetically distinct populations of *P. syringae* pv. *actinidiae* which, to different extents, infect *Actinidia* spp. worldwide [2]. One of these four populations is responsible, as mentioned above, for the severe outbreak of bacterial canker, which is considered as a pandemic disease. In these cases, in addition to methods for the detection of the pathovar, it is important to identify the virulent population to prevent possible new outbreaks [11].

The improvement and harmonization of detection and diagnosis procedures are of primary importance to coordinate a timely response to contain the spread of pathogens and guarantee plant biosecurity worldwide.

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Received January 21, 2014; **Accepted** January 22, 2014; **Published** January 25, 2014

Citation: Ilardi V, Loreti S (2014) Plant Biosecurity: Diagnosis in Plant Pathology. Biosafety 3: e143. doi:10.4172/2167-0331.1000e143

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