NOTES ON NEW PATHOGENS ON ORNAMENTAL WOODY SPECIES IN NORTH WESTERN ROMANIA

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Abstract

The trade with woody ornamentals is sustained by public demand for beautiful and elaborate gardens and modified landscapes in the line of a long tradition but is also one of the causes for biotic invasions with plants, pathogens and pests. The paper presents the results of a survey in retail centers and nurseries specialized on ornamental woody plants in the areas of Cluj-Napoca and Oradea (North-Western Romania) concerning the invasive pathogens. The focal plants species selected on the base of sale figures were Buxus sempervirens, Thuja plicata and Prunus laurocerasus. Moist chambers incubation and cultivation permitted the recovery of several important pathogens: Xanthomonas arboricola pv. pruni on Prunus laurocerasuss and P. lusitanica, Volutella buxi on Buxus sempervirens, Erysiphe elevata on Catalpa bignonioides, Pestalotiopsis funerea and Passalora sequoiae on Thuja plicata and Libocedrus decurrens var. aurea. The graph of host ranges for the identified pathogens was generated to illustrate the possible pathways from the identified hosts to other potential cultivated and wild hosts.

Key words: woody ornamentals trade, invasive pathogens, Thuja plicata, Buxus sempervirens, Prunus laurocerasus, Catalpa bignonioides.

INTRODUCTION

One of the most striking anthropogenic impacts on global biodiversity is the trade with ornamental plants which is contributing at unprecedented pace to the plants dispersal well beyond their natural ranges. Human curiosity and aesthetic needs trigger the economic boom of ornamental plants cultivation and commercial expansion. Gardens are considered works of art from the perspective of environmental esthetics which refers to natural but also built in beautiful environments (Kquofi, Glover, 2012). The concept was developed by Carlson (2003) as covering the human aesthetic experience triggered by nature and human artifacts such as gardens, urban green areas, and human modified landscape in conjunction with the philosophy of biodiversity beauty. However, the pleasure of gardening cannot be reduced to mere economic expediency but the ideal garden should be a microcosm and a tribute to local biodiversity rather than a collection of exotic species (Lovelock, 1987). The aesthetic experience with natural world is imprinted in our biological background (Bourassa, 1991) therefore gardens and cultivated landscapes exert such a powerful impact upon our senses.

Nevertheless, gardening and landscaping have generated a new area of concern due to the invasiveness beyond controlled environment of some plant species initially cultivated only in the confines of gardens and due to the expansion of pests represented by pathogens and consumers such as insects, spiders or nematodes mediated by the global plant trade. In Belgium reports show that 93% of invasive plants are available in nurseries and 67% of these are subjected to sale (Halford et al., 2011).

Many pathogens escaped from cultivated ornamentals into the wild were contributing to the decline of several forest woody species (Santini et al., 2012).

The paper presents several invasive or new pathogens observed in retail centers and gardening shops in North-Western Romania, in Oradea and Cluj-Napoca areas in 2013. Considering the fact that the trade with ornamental species is one of the main pathways for the introduction of invasive pathogens and pests, the detection and warning are important in limiting their introduction.

MATERIAL AND METHODS

Observations and collection of diseased material were performed in several retail centers for ornamental plants in city of Cluj Napoca as well as in one nursery in the proximity of the city of Oradea during 2013. The names of trading centers and nursery are not disclosed to avoid any commercial harm to the owners.

Isolates were obtained from the diseased material and cultivated on appropriate media: malt-agar and potato-dextrose-agar for fungi, King and Sabouraud media for bacteria.

Inoculation was performed from suspension of bacteria in sterile water obtained from minced leaf lesions detached from diseased leaves of *Prunus lusitanica* and *P. laurocerasus*. For confirmation of bacterial disease induced by *Xanthomonas arboricola* pv. *pruni*, pathogenicity test was performed by scratching healthy leaves of *Prunus laurocerasus* and inoculating bacteria collected from pure culture.

In fungi, the stimulation of conidia production was induced by incubation in moist chambers; the material was repeatedly washed in tap water, disinfected in alcohol 70% and rinsed in distilled water prior to incubation in sterile moist chambers. *Pestalotiopsis funerea* and *Volutella buxi* were isolated on malt agar and potato dextrose agar media.

For obligate pathogens such as *Erysiphe elevata*, the identification was based on microscopic characters of chasmothecia.

Identification was based on microscopic and culture characters, using information from published papers.

A graph depicting the host range of the identified pathogens, many of them being also present in investigated retail centers was generated using Pajek software (Batajeli, Mrvar, 2010).

RESULTS AND DISCUSSION

Based on the declarations of retailers' personnel, the purchased plant material from Romanian nurseries and imported from several European countries is generally maintained under observation in local quarantine prior to sale display. However, our observations show that there are always plants on display showing disease symptoms. Several important and dangerous pathogens were identified on plants vegetating in retail centers and in the nursery. Based on interviews, the most frequently sold items were *Thuja plicata* and *Buxus sempervirens,* an increasing trend in sales of *Prunus laurocerasus* being observed. The study was therefore focused on the detection of pathogens on these species.

On potted *Buxus sempevirens* L. was identified *Vollutella buxi* (Corda) Berk (teleomorph: *Pseudonectria buxi* (DC.) Seifert, Gräfenhan & Schroers), important and aggressive pathogen traced also in plants cultivated in hedges, parks and gardens in both urban areas. Frequently it is associated with an invasive species causing outbreaks over the world, *Cylindrocladium buxicola* (Šafránková et al., 2012; Hsiang, 2012) not identified so far in our investigation. The main sources of potted plants were Romanian nurseries, mostly in the proximity of Cluj-Napoca and Oradea. Hosts for this oligophagous opportunistic species are placed in family Buxaceae, mainly on *Buxus* spp. and *Sarcococca* spp.: the origin of the pathogen remains still unknown. At the present moment it causes outbreaks in Europe, North America and New Zealand. The main symptoms consist of leaves' discoloration; first the leaves are yellowing, with time become tan colored, lie close to the stem and turn upward. On the lower surface, creamy-pink setose sporodochia are developing (Photo 1, D). Spores are ellipsoidal and hyaline, conidiophores branched, phialides hyaline, setae unbranched and hyaline (Seifert and Gams, 2011) (Photo 1, C). Other symptoms affect the wood which is discolored under the bark in gray bluish tones. The infections occur through clipping wounds, the species being opportunistic and attacking stressed plants.

Xanthomonas arboricola pv. *pruni* (Smith) Vauterin et al. was identified on potted *Prunus lusitanica* L. (Photo 1, E) and *Prunus laurocerasus* L. imported from Holland where recently *Prunus laurocerasus* was reported as new host for this pathogen (Tjou-Sin et al., 2012) first mention on *Prunus laurocerasus* being reported in France (EPPO RS 97/112). The species is a quarantine EPPO/IIA2 (OEPP/EPPO, 1978)

organism, with a worlwide distribution, currently spreading in Europe. The main symptoms include leaf spots and shot-holes, twig dieback, stem and shoot cankers, leaf deformation. It is a Gram negative motile rod, of 0.2-0.8x0.8-1.7µm (Hayward, Waterston, 1965). The host range includes species from wild and cultivated *Prunus* spp. The dissemination pathways are not known at the moment (Bergsma-Vlami et al., 2012).

On microscopic slides, sections through characteristic lesions revealed the bacterial stream. On King and Sabouraud media (Photo 1, G), the colonies developed from detached lesions washed and surface sterilized then suspended in sterile distilled water were pale cream, translucent, slimy, characteristic for *Xanthomonas* spp.cultures (Schaad et al., 2001). Pathogeneity test developed on detached, healthy leaves of *Prunus laurocerasus* yielded discolororation reactions at the site of inoculation in 2-3 days. In lesions, as opportunistic species developed *Epicoccum purpurascens* Ehrenb.

Pestalotiopsis funerea (Desm.) Steyaert was identified in nursery cultivated *Thuja plicata* Donn. ex D.Don and in potted plants displayed in the retail center. It was repeatedly identified in nurseries as well as in *Thuja plicata* cultivated in hedges, parks and gardens in Oradea and Cluj-Napoca. The species is polyphagous attacking mostly coniferous species but it was also described from *Quercus pyrenaica* Willd. (Bajo et al., 2008) producing leaf and twig blight and stem cankers. The isolation and cultivation were performed on malt agar medium. *Pestalotiopsis funerea* is a cosmopolitan and polyphagous species producing foliage blight, twig dieback, stem cankers in seedlings and adult trees. The disease is more severe in seedlings which may result in high mortality (Maharachchikumbutan et al., 2011). It is an aggressive pathogen in stressed plants or an opportunistic species, the damage being extended in densely cultivated plants. The stress can be induced by a previous attack of cypress aphid (*Cynara cupressivora*), also an invasive species, or root pathogens (*Phytophthora* spp.), soil condition such as drought, waterlogging or wind damage. Colonies developed on malt agar were creamy white, dull cream on reverse, cottony, with moist brown-black, shiny masses of conidia (Photo 1, B). Microscopy confirmed the identification. Conidia are 5 celled, brown middle cells with hyaline marginal cells and 3 characteristic appendages at one end and one at the opposite end (Photo 1, A). The pathogen was also identified on displayed *Libocedrus decurrens* Torr. var. *aurea* imported from Italy.

Passalora sequoiae (Ellis & Everh.) Y.L. Guo & W.H. Hsieh (synonymous to *Cercospora sequoiae*) is an invasive pathogen in Europe, producing leaf spots on foliage of species from Cupresaceeae family (Petersen, 1977; Mathurin, 2010). The foliage turns brown in centripetal

direction, one of most important diagnostic symptoms. Eventually, the diseased trees and shrubs die off. In our observations, diseases potted *Thuja plicata* vegetating in retail center exhibited symptoms and developed on leaves specific black-brown conidiomata of the pathogen after incubation in moist chambers,. Microscopic characteristics include geniculate, brown conidiophores of 50-125x4-6 µm and cilindrical, slightly tapering, 5-6 septate, echinulate, yellow-brown conidia of 405x5.5 µm (Crous and Braun, 2003).

It was associated with *Pestalotiopsis funerea* on same plants.

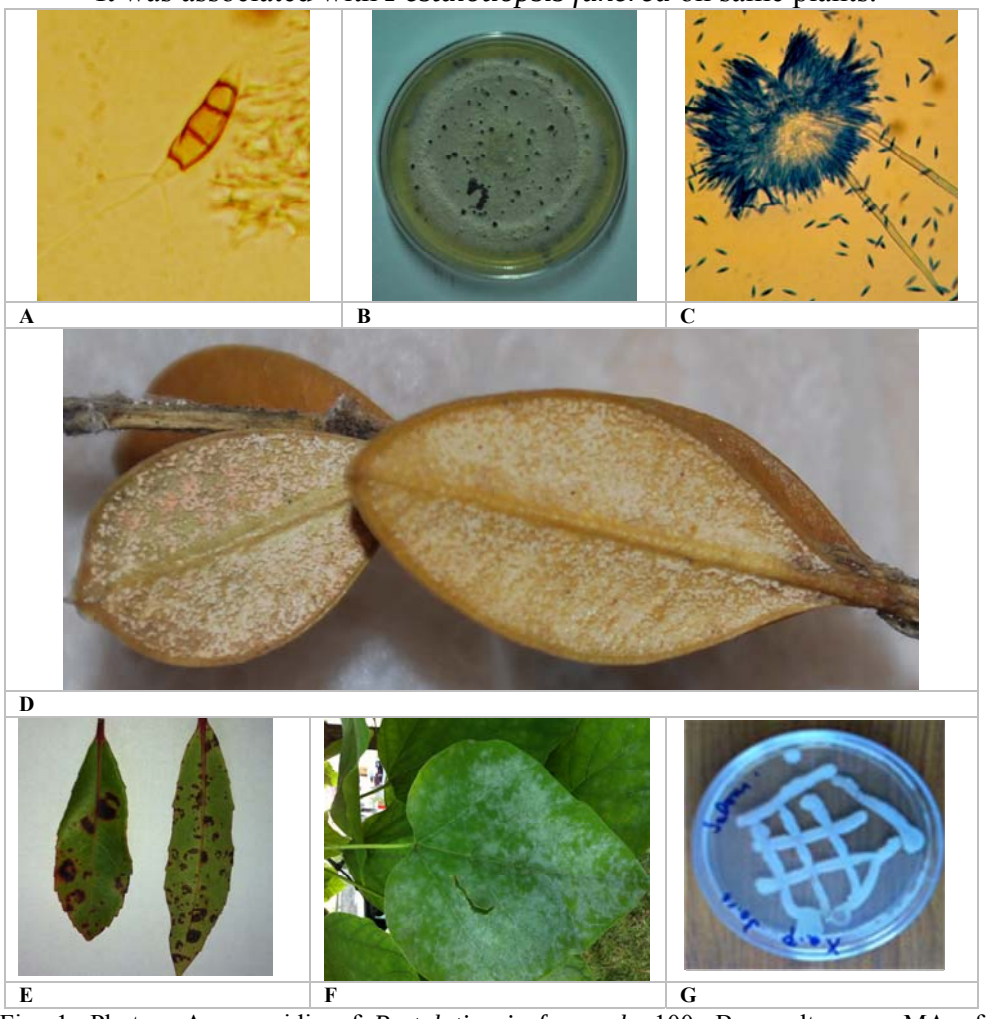

Fig. 1. Photos: A – conidia of *Pestalotiopsis funereal* x100: B – culture on MA of *Pestalotiopsis funerea*: C – setae, phialoconidia, groups of conidiphores with phialides x40 of *Volutella buxi* : D – sporodochia of *Volutella buxi* on leaf underside: E – lesions of *Xanthomonas arboricola* pv. *pruni* on *Prunus lusitanica*: F – *Erysiphe elevata* on leaves of *Catalpa bignonioides* G – culture of *Xanthomonas arboricola* pv. *pruni* on Sabouraud medium (Photos O. Hâruța).

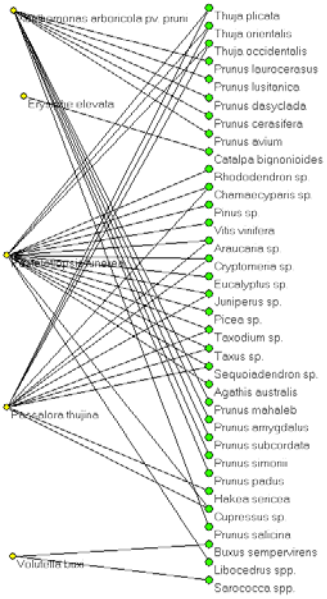

Fig. 2. Network of hosts of the identified pathogens on woody ornamentals in monitored retail centers.

On *Catalpa bignonioides, Erysiphe elevata* (Burrill) U.Braun & S. Takam was identified in potted plants displayed for sale. During the last three years, in hedges and parks in Oradea and Cluj-Napoca, *Catalpa bignonioides* trees presented characteristic foliar symptoms of this new, invasive pathogen, originating from North America (Braun, 1995) recently extending in Europe (Vajna et al., 2004). The anamorph *Pseudoidium* type with elliptical terminal conidia developing during the summer is covering with white powdery bloom the upper surface of the leaves of Indian bean tree. Chasmothecia develop on the upper side of leaves in the autumn presenting dichotomously branched appendages containing short stalked or sessile asci of 45-55 × 35-40 µm with ovoid, hyline ascospores of 20-25 × 12-14 µm (Pastirčáková et al., 2006; Denchev, 2008).

The network (Fig. 2) depicts the host range of the identified pathogens which illustrates the spreading of pathogens to new hosts from sold diseased plants. Many of attached species can be purchased from the same retail centers. The network constructed on the range of hosts shows that *Pestalotiopsis funerea* and *Passalora sequoiae* share almost same hosts. Among wild species susceptible to infection with *Pestalotiopsis funerea* are *Juniperus* spp., *Pinus* spp, *Rhododendron* spp., *Taxus* spp., in one of the visited retail centers being found on potted *Libocedrus decurrens* var. *aurea*. *Xanthomonas campestris* pv. *pruni* recovered from potted *Prunus*

lusitanica and *P. laurocerasus* is a serious menace to wild and cultivated species of stone fruits from genus *Prunus*, the risk is ever greater in the absence of informations on pathogen dispersal pathways.

Besides pathogenic species, there are interesting other species such as mycorrhizal emerging in the pots containing exotic imported ornamentals. It is the case of *Arbutus unedo* imported from Italy showing a carpophore of *Laccaria bicolor*, a common mycorrhizal species associated with strawberry tree among other *Laccaria* species (Garcia et al., 2011). Mycorrhizal species are no doubt, beneficial organisms however, there is always a risk to introduce species that become invasive such being the case of the introduction of two mycorrhizal species in New Zealand associated with cultivated *Pinus contorta*, namely, *Amanita muscaria* and *Suillus luteus* (Dickie et al., 2008).

The diverse collection of woody plants displayed in retail centers is an artificial assembly which harbors pathogens capable of infecting a wide range of hosts unlikely to be possible in natural environments and generate pathways leading pathogens to wild host in urban areas and then, in natural ecosystems if there are susceptible hosts. Apparently quarantine measures are not enough to secure the trade with ornamentals againsts pathogens and pests.

Polyphagous as well as oligophagous pathogens are hazardous and can penetrate cultivated as well as wild environments. For instance, *Pestalotiopsis funerea* is spreading in urban areas as well as *Erysiphe elevata*, their hosts being largely employed in parks, hedges and private gardens. *Xanthomonas arboricola* pv. *pruni* is particularly dangerous affecting orchard species, wild and ornamentals as well.

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