First Report of *Pseudomonas savastanoi* pv. *nerii* on Oleander in the Czech Republic

Václav KŮDELA[1], Ivana ŠAFRÁNKOVÁ [2], Václav KREJZAR[1] and Josef KORBA[1]

[1]Department of Bacteriology, Division of Plant Medicine, Research Institute of Crop Production, Prague-Ruzyně, Czech Republic; [2]Faculty of Agriculture, Mendel University of Agriculture and Forestry Brno, Czech Republic

Abstract

Kůdela V., Šafránková I., Krejzar V., Korba J. (2005): **First report of** *Pseudomonas savastanoi* pv. *nerii* **on oleander in the Czech Republic**. Plant Protect. Sci., **41**: 33–37.

The bacterium *Pseudomonas savastanoi* pv. *nerii* was identified as the causal agent of parenchymatous galls on leaves of potted oleander plants grown at Brno, Moravia, in 2004. The plants had originated from cuttings made from firm shoots of a supposedly asymptomatic plant grown in and introduced from the Mediterranean region. The Biolog GN microplate system was used to identify the isolated bacterial strains. Successful inoculation of *Nerium oleander* seedlings proved the pathogenicity of the isolates. This is the first record of *P. savastanoi* pv. *nerii* in the Czech Republic.

Keywords: bacterial knot of oleander; *Pseudomonas savastanoi* pv. *nerii*; *Nerium oleander*; Czech Republic

In the Czech Republic, oleander (*Nerium oleander* L.) is a popular ornamental woody plant grown in castle gardens, spa parks, door-yard plantings or balconies. Since oleander requires a minimum winter temperature of about 4–8°C it is not suitable for cultivation outdoors in this country. Therefore, potted plants are grown outdoors only during summer. In early fall the oleanders are returned to greenhouses or other indoor facilities for the winter.

In spring 2004, we obtained a sample of oleander leaves with small round galls (knots, outgrowths) on their surfaces (Figure 1). They came from potted oleander plants grown at Brno, Moravia. The plants originated from cuttings made from firm shoots of a supposedly asymptomatic plant originally raised in the Mediterranean region.

The leaf galls on the oleander sample resembled bacterial knot. Similar diseases occur on common ash (*Fraxinus excelsior* L.), olive (*Olea europea* L.), privet (*Ligustrum japonicum* Thunb.) and species of *Jasminus, Forsythia,* and *Phyllyrea* among *Oleaceae* (Iacobellis 2001). The pathogens of these diseases had been classified under different names over the years. Recently, strains isolated from oleander (*Apocynaceae*), from ash and from olive and other *Oleaceae* have been classified, respectively, as *P. savastanoi* pv. *nerii*, *P. savastanoi* pv. *fraxini*, *P. savastanoi,* and *P. savastanoi* pv. *savastanoi* (Young 1996). Bacteria isolated from different host plants differ in host specificity. Oleander strains infect oleander and plants of *Oleaceae*, whereas olive strains infect *Oleaceae.*

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Strains from ash specifically infect only ash plants (Janse 1982; Iacobellis *et al*. 1998).

In spite of the fact that pathovars of *P. savastanoi* prefer warmer climates, the bacterial canker of ash is of economic importance for the Czech Republic. It is widespread in the Elbe river basin and in Bohemia and southern Moravia (Uroševič 1976). Nevertheless, there is no record on occurrence of bacterial knot of oleander in the Czech Republic. In some instances the tumors produced artificially by *Agrobacterium tumefaciens* (= *Rhizobium radiobacter*) are in their youngest stages very similar to the young natural knots (galls) on oleander caused by knot bacterium (Smith 1928). Therefore, we decided to determine the cause of the leaf galls on oleander plants grown in this country, to specify the strains causing these galls, and to assess the epidemiological ramifications of this occurrence.

MATERIAL AND METHODS

The isolation of suspected bacteria was carried out on King et al's medium B, using standard isolation techniques (Schaad *et al*. 2001). Galls from oleander leaf laminae were surface sterilised with 70% ethanol. Pieces of gall tissues were placed in a droplet of sterilised water in a flamed watch glass and mechanically crushed. After 20 min a loopful of macerate was streaked onto nutrient agar in Petri dishes and incubated at 26°C. Colonies of bacteria were re-streaked to obtain pure cultures of representative strains.

The Biolog Identification System GN MicroPlate system (developed by Biolog, Inc., Hayward, USA) was used to identify the isolated bacterial strains. The test yields a characteristic metabolic fingerprint. The microplates were incubated for 4 and 24 h. Biolog's MicroLog 2 4.2 software was used to identify the bacterium from its metabolic pattern. The calculations for the identification of bacteria as to genus, species and other taxonomic units are based on a similarity index (Schaad *et al*. 2001).

The pathogenicity of eight bacterial isolates was tested on 8-month-old seedlings of *Nerium oleander* (unknown cultivar) using two plants per isolate. Plants were inoculated by injection with a hypodermic needle of a 10^7 cells/ml suspension of a 2-day nutrient agar culture in sterilised water. Young stems were injected at the leaf axils, and leaf laminae both in the midrib and intervenal areas. For control, stems and leaves were injected with sterilised water. Plants were kept in a greenhouse at 24 to 26°C and observed for symptom development up to 3 months after inoculation.

RESULTS AND DISCUSSION

Bacterial strains that were later determined as *Pseudomonas savastanoi* pv. *nerii* (hereafter referred to as pv. *nerii*) were easily isolated from fresh parenchymatous galls showing lysogenous cavities (Figure 2). However, galls having a necrotic appearance were predominantly occupied by secondary organisms, e.g. by oxidase-positive non-fluorescent pseudomonads.

Twelf of the oxidase-negative strains from oleander were classified using the Biolog Identification System. The results presented in Table 1 show that five strains were *Pseudomonas syringae* pv. *nerii*,

Figure 1. Parenchymatous knots on leaf surface of oleander. Natural infection (Photo I. Šafránková)

Figure 2. Cross section of parenchymatous knot showing lysogenous cavities with bacterial slime (Photo V. Krejzar)

five were *Pseudomonas* sp., and one strain was *P. savastanoi* pv. *glycinea*. One strain, isolate 05, was classified as *P. savastanoi* pv. *nerii* after 4 h incubation time and as *P. savastanoi* pv. *glycinea* after 24 h incubation time.

When bacterial isolates, determined as pv. *nerii*, were inoculated into young oleander plants, they induced slight galls (outgrowths) around the sites of inoculation. Secondary galls (outgrowths) were also produced outside the sites of inoculation after secondary spread of the bacteria. On young stems, a clump of galls appeared within diseased tissues (lesions) causing it to split (Figure 3).

Some of the bacterial isolates did not cause conspicuous galls on young stems. Instead, they induced dark brown to blackened lesions. The leaves on such stems were bent towards the stem and leaf laminae had inrolled margins (Figure 4).

Forty days after inoculation, bacteria were reisolated from infected oleander tissues and compared with the original cultures used for inoculation. Each re-isolate of pv. *nerii* had retained the characteristics of the isolate initially used for inoculation.

We can conclude that the presence of pv. *nerii* has been confirmed as the cause of parenchymatous galls on leaves of potted oleander grown at Brno, Moravia. Earlier, the pathogenicity of gall-causing strains from ash, oleander and olive had been examined Urošević (1976). Of the strains of *Pseudomonas savastanoi* tested in his work, only the strains from ash had come from the Czech Republic. Strains from olive and oleander plants had originated in former Yugoslavia.

How might pv. *nerii* have been introduced to the Czech Republic, and are these strains a threat to ash? Knowing the history of the oleander plants on which pv. *nerii* was found in the Czech Republic, it seems probably that the pathogen was introduced on firm shoots that had been cut from an asymptomatic plant grown in the Mediterranean region. This kind of transmission is not surprising because the related bacterium *Pseudomonas savastanoi* pv.

Figure 3. Primary and secondary parenchymatous knots following inoculation with *Pseudomonas syringae* pv. *nerii* on olender shoot (Photo V. Krejzar)

Figure 4. Dark brown to blackened lesion on young oleander stem and downward curling of leaves following inoculation with *Pseudomonas syringae* pv. *nerii* (Photo J. Šillerová)

Table 1. Identification of bacterial isolates from parenchymatous knots on oleander plants using Biolog system

Isolate	Name of bacterium	Incubation time (h)	Similarity index	Probability (%)
03		24	0.79	86
06		24	0.72	83
09	*Pseudomonas savastanoi* pv. *nerii*	24	0.59	69
012		24	0.54	61
07		24	0.51	61
05	*Pseudomonas savastanoi* pv. *nerii*	4	0.87	95
	Pseudomonas savastanoi pv. *glycinea*	24	0.53	71
010	*Pseudomonas savastanoi* pv. *glycinea*	24	0.53	76
08				
01				
011	*Pseudomonas* sp.	24	ND	ND
02				
04				

ND – not determined

savastanoi is commonly found on the phylloplane of the olive (Ercolani 1985). Therefore, the use of pathogen-free propagating materials (cuttings etc.) is recommended to exclude the pathogen from host plants (Iacobellis 2001).

In the Czech Republic, the economic importance of bacterial knot on oleander is probably minimal, because this ornamental woody plant is grown on a small scale. For the same reason, pv. *nerii* strains represent only a minor risk as a source of infection for ash. Strains of pv. *fraxini* from ash are not able to infect oleander plants.

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Abstrakt

Kůdela V., Šafránková I., Krejzar V., Korba J. (2005): **První výskyt bakterie** *Pseudomonas savastanoi* pv. *nerii* **na oleandru v České republic**e. Plant Protect. Sci., **41**: 33–37.

Bakterie *Pseudomonas savastanoi* pv. *nerii* byla v roce 2004 identifikovaná jako původce parenchymatických hálek na listech oleandru pěstovaném v Brně. Původce byl izolován z hálek na listech oleandru, který pocházel ze stonkových řízků odebraných ze zjevně bezpříznakové rostliny pěstované ve Středomoří. K identifikaci izolovaných bakteriálních kmenů byl použit systém Biolog GN. Patogenita bakteriálních izolátů byla prokázána inokulací semenáčků *Nerium oleander*. Poprvé tak byl prokázán výskyt *Pseudomonas savastanoi* pv. *nerii* na území České republiky.

Klíčová slova: parenchymatické listové hálky; *Pseudomonas savastanoi* pv. *nerii; Nerium oleander;* Česká republika

Corresponding author:

Prof. Ing. Václav Kůdela, DrSc., Výzkumný ústav rostlinné výroby, odbor rostlinolékařství, oddělení bakteriologie, 161 06 Praha 6-Ruzyně, Česká republika
tel.: + 420 233 024 427, fax: + 420 230 310 636, e-mail: kudela@vurv.cz