THE IMPORTANCE OF THE TYPE OF *Erwinia amylovora* INOCULUM IN SCREENING OF APPLE GENOTYPES SUSCEPTIBILITY TO FIRE BLIGHT

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A B S T R A C T

Terminal shoots of 3 apple cultigens ('Free Redstar'. 'Ligolina' and J-79) growing in the greenhouse, were inoculated with 2 types of inoculum: either a mixture of 4 *E. amylovora* strains or with each strain separately. The strains were isolated from apple, pear or hawthorn plants in various regions of Poland. Differences in virulence level among strains were found. However, there was not a case where the same strain showed different virulence with the different genotypes inoculated. The severity of fire blight was reflected by type of inoculum and virulence of strain used.

Key words: *Malus*, breeding, fire blight, resistance evaluation, single strain inoculum, mixture of strains inoculum

INTRODUCTION

The susceptibility of apple and pear genotypes to fire blight is usually determined by artificial inoculations of terminal shoots and/or blossoms with *E. amylovora.* Generally, strains of this bacterium are not host species-specific, i.e. strain originating from apple is pathogenic on pear, hawthorn and other host plants (Vanneste, 1995). However, strains from *Rubus* species showed such specificity (Heimann and Worf, 1985; Ries and Otterbacher, 1977). The differences in virulence among *E. amylovora* strains have been reported (Cabrefiga and Montesinos, 2005; Fazio et al., 2006, 2008; Lespinasse and Aldwinckle, 2000; Norelli et al., 1984, 1987; Paulin et al., 1993; Puławska et al., 2006; Shaffer and Goodman, 1962). For screening genotype's susceptibility

to fire blight some authors consider using inoculum containing a mixture of several highly virulent strains originating from various hosts as more reliable and more reflective of the situation occurring in nature where cross infections are common (Lespinasse and Aldwinckle, 2000; Norelli et al., 1987, 2003). Others think that mixing of strains might lead to various interactions between them resulting in a decrease of disease amount after inoculation (Paulin and Lespinasse, 1990).

The purpose of the study presented was to determine the possible role of single or mixed *E. amylovora* inoculum in evaluating susceptibility of apple cultigens to fire blight.

MATERIAL AND METHODS

Experiments were conducted during 2005 and 2006 on trees of 3 apple cultigens: 'Free Redstar', 'Ligolina' and J-79, hand grafted on M.9, growing in pots in the greenhouse. Terminal shoots were inoculated by cutting-off their tips just below the first undeveloped leaf using scissors previously immersed in water suspension of a single strain or a mixture of 4 strains of *E. amylovora* (in both cases at 10^7 cfu/ml) originating from various fire blight hosts: Ea 650 and Ea 684 (hawthorn), Ea 659 and Ea 694 (apple) and Ea 613a (pear) and deposited at own collection. In 2005, Ea 650, Ea 684 and Ea 659 and Ea 694 were used and in 2006 Ea 650, Ea 659, Ea 694 and Ea 613a. The mixture was prepared by combining equal volumes of water suspension of each strain at 10^7 cfu/ml. The pathogenicity of the strains was proved before experiments on terminals of apple Antonovka seedlings and pear fruitlets according to the methods described by Sobiczewski and Millikan (1985) and Sobiczewski et al. (2004). Observations and measurements of the progress and severity of fire blight were performed 2, 4 and 6 weeks after inoculation. Each cultigen and combination was represented by 20 trees in 2005 and 16 trees in 2006. The results were subjected to an analysis of variance ANOVA with means separation using Neuman-Keuls test at 5% level of significance. The disease severity was expressed as percentage of necrosis in relation to entire length of a shoot and as disease susceptibility score according to the scale of Le Lezec et al. (1997), where: 1 – very low susceptible (0-20% of shoot length necrotized); 2 – low susceptible (> 20-40%); 3 – moderately susceptible (> 40-60%); 4 – susceptible (> 60-80%) and 5 – very susceptible (> 80-100%).

RESULTS AND DISCUSSION

In the first year of study, no differences in severity of fire blight as related to type of inoculum were found (Tab. 1). Using one strain (Ea 659) inoculum only and a mixture of 4 strains gave similar amount of disease on all cultigens tested. However, their various susceptibility was found: 'Free Redstar' appeared to be the most resistant, 'Ligolina' the most susceptible and J-79 showed medium susceptibility. These results confirmed our earlier findings (Sobiczewski et al.,

Table 1. Apple shoots susceptibility to fire blight after inoculation with a single *Erwinia amylovora* strain or a mixture of 4 strains (2005)

Cultigen/Inoculum	Percentage of shoot necrosis lesion after:		
	2 weeks	4 weeks	6 weeks
Free Redstar			
Single strain (Ea 659)	10.5 a**A***	12.8 aA	13.2 aA (1)****
Mixture of 4 strains*	8.7 aA	10.2 aA	10.6 aA (1)
J -79			
Single strain (Ea 659)	21.1 aB	23.8 aB	25.3 aB (2)
Mixture of 4 strains*	24.8 aB	27.4 aB	29.5 aB (2)
Ligolina			
Single strain (Ea 659)	35.2 aC	66.4 aC	71.2 aC (4)
Mixture of 4 strains*	34.2 aC	62.3 aC	70.9 aC (4)

* Mixture of 4 strains: Ea 650 and Ea 684 (hawthorn), Ea 659 and Ea 694 (apple)
**values with the same small letter indicate no difference within the same cultigen
***values with the same capital letter indicate no difference between cultigens (P = 0.05)
****Susceptibility classes (Le Lezec, 1997)

Table 3. Apple shoots susceptibility to fire blight after inoculation with a single strain of *Erwinia amylovora* or a mixture of 4 strains (2006)

Cultigen/Inoculum	Percentage of shoot necrosis lesion after:		
	2 weeks	4 weeks	6 weeks
Free Redstar			
Ea 650	3.2 a	5.0 a	6.5 a (1)
Ea 659	7.6 b	13.0 b	14.0 ab (1)
Ea 694	13.3 c	22.0 c	23.2 c (2)
Ea 613a	10.0 bc	20.3 bc	19.5 bc (1)
Mixture of 4 strains*	9.3 b	12.7 b	14.0 ab (1)
Ligolina			
Ea 650	39.9 a	57.3 a	63.4 a (4)
Ea 659	48.6 ab	56.0 a	64.6 a (4)
Ea 694	52.3 b	68.9 b	75.4 bc (4)
Ea 613a	47.1 ab	70.1 b	79.6 c (4)
Mixture of 4 strains	52.3 b	68.2 b	71.6 b (4)

*Mixture of strains: Ea 650 (hawthorn), Ea 659 and Ea 694 (apple), Ea 613a (pear)
For other explanations see Table 1

2004; 2006) from experiments with application of single strain inoculum. In the second year, trees of 2 cultigens ('Free Redstar' & 'Ligolina') inoculated either with a mixture of 4 strains or with each of them separately showed some differences in strain virulence, which was reflected in severity of fire blight caused by mixed inoculum (Tab. 3). On both cultigens, the strain showing a lower virulence level influenced on decrease of disease severity after inoculation with the mixture in which this strain was used. There was not a case where the same strain showed different levels of virulence with the different genotypes used. Quamme and Bonn (1981) also found no evidence of significant cultivar x strain interaction when the virulence of nine strains of *E. amylovora* was compared on four cultivars of pear. In contrast, study of Norelli et al. (1984) clearly indicate that differential interactions occur between apple cultivars and strains of *E. amylovora.* On the other hand, Paulin and Lespinasse (1990) pointed out that using the mixture of strains for inoculation of apple shoots did not always give a higher overall disease incidence and severity than the most virulent strain alone.

In our study, the distribution of individuals of each genotype amongst susceptibility classes reflected their general susceptibility (Tab. 2, 4). In both experiments almost all of 'Free Redstar' individuals belonged to class 1 (grouping plants with low susceptibility) while almost none of the individuals of 'Ligolina' were classified in this class. However, Norelli et al. (1987) found that inoculation of greenhouse-grown apple seedlings with a mixture of five strains of *E. amylovora* resulted in a larger percentage of seedling population being evaluated as susceptible, than when the seedlings were inoculated with a single strain. In our study this tendency was not observed. At present, many researchers apply for the genotype screening tests inoculum made from single, highly virulent strain of *E. amylovora,* however it is very important to select of the such strain. Cabrefiga and Montesinos (2005) documented that more aggressive (*sensu* more virulent) strain showed the higher rate of disease progression and shorter disease incubation period at a lower inoculum concentration than less aggressive strain. The differences in virulence among *E. amylovora* strains can influence reliable evaluation of particular genotype to fire blight (Norelli et al., 2003; Fazio et al., 2006, 2008). The study on biodiversity of this pathogen showed that strains originating from North America, where fire blight was described for the first time, are generally more genetically heterogeneous than those from Europe (Giorgi, Scortichini, 2005; Jock et al., 2002; Jock, Geider, 2004). It was also found that *E. amylovora* isolates can differ in plasmid content. Some of plasmids, like pEI70 discovered in Spain, can posses genes essential for pathogenicity (Llop et al., 2008), different than presently known ones, which are responsible for synthesis of siderophores, exopolysaccharides

Table 2. Distribution of individuals of apple cultigens (%) amongst susceptibility clasess (2005)

Cultigen/Inoculum	Susceptibility classes**														
	1			2			3			4			5		
	evaluation after (weeks)														
	2	4	6	2	4	6	2	4	6	2	4	6	2	4	6
Free Red Star															
Single strain (Ea 659)	85	80	75	15	20	25	0	0	0	0	0	0	0	0	0
Mixture of 4 strains*	90	95	95	10	5	5	0	0	0	0	0	0	0	0	0
Ligolina															
Single strain (Ea 659)	20	5	5	50	0	0	20	21	10	10	64	63	0	10	22
Mixture of 4 strains*	5	0	0	65	10	0	30	20	15	0	65	70	0	5	15
J-79															
Single strain (Ea 659)	50	45	45	45	40	45	5	15	10	0	0	0	0	0	0
Mixture of 4 strains*	30	25	25	60	60	50	10	15	25	0	0	0	0	0	0

*Mixture of 4 strains: Ea 650 and Ea 684 (hawthorn), Ea 659 and Ea 694 (apple)

Table 4. Distribution of individuals of apple cultigens (%) amongst susceptibility clasess (2006)

Cultigen/Inoculum	Susceptibility classes														
	1			2			3			4			5		
	evaluation after (weeks)														
	2	4	6	2	4	6	2	4	6	2	4	6	2	4	6
Free Red Star															
Ea 650	100	94	81	0	6	19	0	0	0	0	0	0	0	0	0
Ea 659	94	94	88	6	6	12	0	0	0	0	0	0	0	0	0
Ea 694	75	62	50	25	25	44	0	13	6	0	0	0	0	0	0
Ea 613a	94	69	69	6	13	6	0	18	25	0	0	0	0	0	0
Mixture of 4 strains*	94	88	88	6	12	12	0	0	0	0	0	0	0	0	0
Ligolina															
Ea 650	6	0	0	38	0	0	56	62	44	0	38	56	0	0	0
Ea 659	0	0	0	19	0	0	75	81	19	6	19	75	0	0	6
Ea 694	0	0	0	0	0	0	81	18	6	19	63	63	0	19	31
Ea 613a	0	0	0	19	0	0	75	25	6	6	44	38	0	31	56
Mixture of 4 strains*	0	0	0	19	0	0	56	24	18	25	63	69	0	13	13

*Mixture of strains: Ea 650 (hawthorn), Ea 659 and Ea 694 (apple), Ea 613a (pear)

and proteins e.g. harpin. It seems that the problem needs more detailed study to develop standardized method, especially for breeding purposes.

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ZNACZENIE RODZAJU INOKULUM *Erwinia amylovora* W BADANIACH NAD OCENĄ PODATNOŚCI GENOTYPÓW JABŁONI NA ZARAZĘ OGNIOWĄ

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S T R E S Z C Z E N I E

Młode pędy (przyrosty) na drzewkach 3 genotypów jabłoni (‘Free Redstar’. ‘Ligolina’ i J-79), rosnących w pojemnikach w szklarni, zakażano 2 rodzajami inokulum: mieszaniną 4 szczepów *E. amylovora* lub każdym szczepem oddzielnie. Szczepy pochodziły z jabłoni, gruszy i głogu z różnych rejonów Polski. Stwierdzono różnice w wirulencji szczepów, jednak żaden z nich nie wykazał zróżnicowania wirulencji w stosunku do badanych genotypów. Nasilenie zarazy ogniowej na zainokulowanych pędach odzwierciedlało rodzaj zastosowanego inokulum oraz stopień wirulencji szczepów.

Słowa kluczowe: *Malus*, hodowla, zaraza ogniowa, ocena odporności, inokulum mieszane, inokulum pojedyncze