Response of selected almond varieties and sélections to Pseudomonas amygdali, the causal agent of the "hyperplastic bacterial canker" of almond

Psallidas P., Stylianides D.K.

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Response of selected almond varieties and selections to *Pseudomonas amygdali*, the causal agent of the « hyperplastic bacterial canker » of almond

P.G. PSALLIDAS & D.C. STYLIANIDES
Pomological Institute, Naoussa (Greece)

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ABSTRACT

The response to artificial inoculation with the bacterium Pseudomonas amygdali, **the cause of the** 'hyperplastic Bacterial canker', **of selected almond varieties, clones and individual trees was investigated.**

Fourteen almond varieties including six (6) Greek varieties and four (4) clones were inoculated artificially through the leafscars and wounds of the bark.

From the varieties tested, Marcona, Cristomorto, Ferraduel and Ferragnes, in descending order exhibited a good degree of resistance. The varieties Texas, Pagrati and Nonpareil exhibited a moderate degree of resistance and they are considered as tolerant. The varieties Furnat de Brezenaud, Troito, Retsou, Papadopoulou, Vavatsikou, Syllogistou and Triconi No 2 as well as the four clones tested were highly susceptible.

The possible source of resistance genes in some varieties and the fate of the resistance are discussed.

RESUME

REPONSE DE CERTAINES VARIETES D'AMANDIERS AUX ATTAQUES DE PSEUDOMONAS AMYGDALI AGENT CAUSAL DU «CHANCRE HYPERPLASTIQUE BACTERIEN» DE L'AMANDIER

La réponse de diverses variétés d'amandiers aux inoculations avec Pseudomonas Amygdali **a été observée.**

Quatorze variétés d'amandes comprenant 6 variétés grecques et 4 présélections ont été inoculées artificiellement sur des cicatrices foliaires ou des blessures d'écorce.

'Marcona', 'Christomorto', 'Ferraduel' **et** 'Ferragnès' **présentent une résistance décroissante mais de bon niveau.** 'Texas', 'Pagrati' **et** 'Non Pareil' **sont moyennement résistantes et peuvent être considérees comme tolérantes.** 'Fournat de Brezenaud', 'Troito', 'Retsou', 'Papadopoulou', 'Vowatsikou', 'Syllogistou' **et** 'Triconi n.° 2' **ainsi que les quatre présélections sont très sensibles.**

L'origine de la résistance génétique de certaines variétés et la forme de la résistance est discutée.

The disease known as «hyperplastic bacterial canker» of almond *(Prunus dulcis)* is the most destructive bacterial disease of this tree in Greece.

The disease was described in 1968 by Psallidas and Panagopoulos who proved the bacterial nature of a long existing disease at Mirabelon area of Crete. Since then the disease has been found in the Aegian islands of Kos, Rhodes, Chios where it is endemic. It has also been found in one orchard in the island of Euboea and in the mainland in the Attiki district.

Outside Greece the disease has been reported in Turkey, in Egee region and it also occurs in Afganistan.

Because of this disease the cultivation of almonds in the above mentioned areas of Greece has declined and most of the orchards have been abandoned.

The characteristic symptom of the disease is the formation of swollen cankers on branches twigs and trunks.

Usually the cankers begin from leaf scars but any wound can serve as an entrance for the pathogen. The first symptoms appear in late winter as the dormacy breaks, as a swelling of the bark in the place around the leaf scars of the last year's vegetation. Later a longitudinal crack appears on the swollen bark extending down to the wood tissue. The affected bark tissues split apart and open cankers 0.5-2.0 cm long are formed. The cankers are surrounded by swollen, rough, dark brown margins. In section the thickened cortical tissues exhibit a soft cheese appearance with some water-soaked spots which turn brown with aging.

As a result of the canker formation the buds corresponding to the affected leaf scar fail to break in the spring or they give new shoots which may die later because of girdling of their base. The so formed cankers are perennial being active not only throughout the year but also for many years. The pathogenic bacterium can be isolated from cankers of any age.

The size of the cankers depends on their age, thus their length on two year old shoots is between 3-5 cm while in older branches and trunks they may reach a length of 15-20 cm.

Cankers resembling those described above can be caused by the fungus *Sclerotinia laxa*, but a careful examination reveals some characteristic differences. In the case of cankers caused by the fungus the margins are also split but their tissues are not swollen and in the middle of the canker the spur is always present and in most of the cases the surface of the canker is covered by the dead bark. On the contrary in the middle of the cankers caused by the bacteria a small hole is evident corresponding to the dead bud.

From epidemiological studies it has been found that the bacterium overwinters inside the cankers and is disseminated in short distances by the rain and wind. In long distances the pathogen is disseminated by infected propagating material.

It has also been found that the bacterium does not live as an epiphyte on leaf surfaces.

Based on the epidemiological data only preventive control methods can be recommended against the disease. These include pruning out diseased parts, uprooting badly affected trees and applying two sprays with copper compounds during the leaf-fall period to cover the leaf scars and to prohibit the entrance of the pathogen.

The use of resistant varieties or selections could be the ideal control method especially for the areas where the disease exists. In these areas almonds are cultivated in slopes of hills with low fertility soils and the application of two sprays is impractical and uneconomic.

So a research to explore the possibility of resistant genes to be found among the known almond varieties as well as among individuals, in the infected areas, seemed worthwhile.

Towards this objective a research programme was scheduled in collaboration with the Institute for Deciduous Fruit Trees of Naoussa.

The programme has two aims:

1) Search for individuals among the diseased trees in infected orchards, which were free from disease symptoms. Such individuals may have acquired some resistance and if so they could be used either to replace the sensitive varieties or as resistance donors in crosses with other sensitive varieties.

2) Evaluation of cultivated or recommended for release, almond varieties for their susceptibility to artificial inoculations with the pathogenic bacterium.

The varieties used in this study are shown in Table I.

As it is shown in this table fourteen (14) varieties were used. Among them there are six (6) Greek varieties.

From a survey in the infected orchards at Mirambelon district, four (4) trees without visible disease symptoms were localized. These trees were marked and used as source for graft-wood and for artificial inoculations.

Three experiments were established in three different locations. One at the Benaki Phytopathological Institute experimental field in Kifissia, the other at the experimental field of the Ministry of Agriculture in Lionetto, Lasithi, Crete and the third in another place in Attiki district. In this third experiment the varieties which exhibited some degree of resistance were tested again in a different location in order to verify the results obtained from the previous experiments.

Two bacterial isolates were used for the artificial inoculations. The isolates AI1 and AI5 isolated from diseased almond trees from Mirambelon, Crete and Chios respectively.

The inoculum was prepared by suspending a 48 hr growth from PDA (Patato Dextrose Agar) slope into 6 ml of sterile distilled water. This suspension has a titre of approximately 2×10^9 c.f.u./ml.

The trees used in the experiments were 3-4 years old and were grafted on bitter almond seedlings. The trees were artificially inoculated in autumn (October-November), time when most of the natural infections take place, although the almond tissues are susceptible to the disease all the year around. Two shoots from each of five trees were used each time. The shoots were 1 to 2 years old and the inoculations were carried out in two ways :

1) By placing a drop of the inoculum on the leaf scars just after detaching the leaves forcibly by hand. Four leaf scars were inoculated on each shoot.

2) By placing a drop of the inoculum suspension into a wound of the bark made with a sterile scalpel. The wounds were then bound with a polythene tape.

The shoots were inoculated in 2 or 3 places beginning from the base of the shoot at 10 cm distance between them.

The results of the inoculations and the progress of the disease were recorded the next spring (April, May) and also the next autumn after the leaf fall.

The different varieties and selections were evaluated for resistance using a scoring system rated from 0 to 3 and based upon the successful establishment of the disease and severity of symptoms : 0 = no symptoms, 1 = small closed inactive looking cankers on the shoots or small swellings of the tissues around the leaf scars, 2 = open cankers on the shoots usually restricted to the inoculation site with swollen margins obviously active. On the leaf scars 1 or 2 small open cankers on the top leaf scars. 3 = well developed open active cankers on the shoots, expanding at both sides of the inoculation site. On the leaf scars at least 2 well developed cankers.

For the evaluation of each variety the mean value of ratings for 10 shoots and corresponding leaf scars was estimated.

The results obtained from the experiments are presented in Table 2.

CONCLUSIONS

From the results obtained it is concluded that most of the almond varieties and the four selections from infected areas are very susceptible to the artificial inoculations with the bacterium *Pseudomonas amygdali.*

The unexpected behaviour of the selections to the artificial inoculations was further investigated and it was found that all of them had an early leaf fall which was completed before the beginning of the rainy season and thus they escape the infections through the leaf scars.

From the varieties tested, *'Marcona', 'Cristomorto', 'Ferraduel'* and *'Ferragnes'* in descending order are considered as having a good degree of resistance.

The varieties *'Texas', 'Pagrati'* and *'Nonpareil'* exhibited a moderate resistance and they are considered as tolerant.

From the results obtained it could be concluded that the varieties *'Ferragnes'* and *'Ferraduel'* may have acquired the resistance genes from their parent variety *'Cristomorto'*, but the other ancestor variety AI has not been evaluated for its resistance to the disease.

From the results presented in the table 2 it is obvious that all the varieties tested gave some response to shoot inoculation. This urged us to investigate further the fate of the bacteria inside these small and sometimes inactive looking cankers. So in the third experiment these cankers were examined carefully and isolations were made from the tissues inside the cankers. This examination revealed that in all cases inside the cankers there is some reaction. All the isolations made from these cankers 1 ½ year after inoculation were positive and the bacterium *P. amygdali* was isolated.

These findings rise some doubts about the fate of the resistance of these varieties because we think that if the growth of the bacterium is not restricted inside the plant tissues the danger the resistance to be broken through the development of new bacterial strains adapted to the new hosts is great.

We consider the results obtained up to now as indicative and we think that the varieties found to possess resistant genes should be tested further under arid conditions as the ones prevailing in the areas where the disease exists.

Table 1

Almond varieties evaluated for their susceptibility to **Pseudomonas amygdali**

A/A	Variety	Origin
1	Ferragnes (CRISTOMORTO x AI)	FRANCE
2	Ferraduel (CRISTOTOMORTO X AI)	FRANCE
3	Marcona	SPAIN
4	Furnat de Brezenaud	FRANCE
5	Nonpareil	USA
6	Cristomorto	ITALY
7	Texas	USA
8	Troito	ITALY
9	Retsou	GREECE
10	Papadopoulou	GREECE
11	Vavatsikou	GREECE
12	Pagrati	GREECE
13	Syllogistou	GREECE
14	Trikono No. 2	GREECE
15	Mirambelo No. 1	GREECE
16	Mirambelo No. 2	GREECE
17	Mirambelo No. 3	GREECE
18	Mirambelo No. 4	GREECE

Table 2

Evaluation of almond varieties and selections for their susceptibility to artificial inoculation with **Pseudomonas amygdali**

Variety	Susceptibility ratings					
	Experiment 1		Experiment 2		Experiment 3	
	Shoot	L. scar	Shoot	L. scar	Shoot	L. scar
Ferragnes			1.2	0.0	1.1	0.1
Ferraduel			0.1	0.0	1.0	0.0
Marcona			0.5	0.0	0.3	0.0
Nonpareil	1.5	0.5				
Cristomorto			1.0	0.0		
Pagrati			1.4	1.0	1.2	0.2
Texas	1.5	0.9				
Troito	2.0	2.1				
F. Brezenaud	2.8	1.5				
Retsou	3.0	3.0			3.0	3.0
Papadopoulou	2.6	2.5				
Vavatsikou			2.8	2.5		
Syllogistou			2.6	3.0		
Trikoni No. 2			3.0	3.0		
Mirambelo 1	3.0	2.8				
Mirambelo 2	3.0	2.5				
Mirambelo 3	2.9	3.0				
Mirambelo 4	3.0	3.0				

Varieties with scores 0-1.2 in shoot inoculations and 0-0.5 in the leaf scar inoculations considered as resistant. With scores 1.2-1.6 and 0.5-1.0 as tolerant. Varieties with higher scores are considered as sensitive.