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# Bacterial diseases of almond rootstocks

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**RESUME** - On décrit le chancre du collet, produit par *Agrobacterium tumefaciens*, la maladie à bactéries la plus répandue et sérieuse pour les porte-greffes d'amandier et pour l'amandier en général. On discute le comportement de quelques porte-greffes de l'amandier vis à vis de l'infection par *Agrobacterium* et la possibilité d'appliquer le contrôle biologique dans la pépinière, avec ses limitations. On décrit aussi le chancre bactérien hyperplastique de l'amandier produit par *Pseudomonas amygdali*. On présente brièvement les maladies causées par la bactérie limitée au xylème, comme phony peach, almond leaf scorch et plum leaf scald.

Mots-clés: Amandier, porte-greffes, bactéries.

**SUMMARY** - Crown gall disease caused by *Agrobacterium tumefaciens* the most common and serious disease of almond rootstocks and almond trees in general is described. The behaviour of some almond rootstocks to infection by the crown gall pathogen and the possibility of applying the biological control method in the nursery practice in order to control the disease and its limitations is discussed. Hyperplastic bacterial canker of almond caused by *Pseudomonas amygdali* is described. Phony peach, almond leaf scorch and plum leaf scald diseases caused by the xylem limited fastidious bacterium *Xylella fastidiosa* are briefly presented.

Key words: Almond, rootstocks, bacterial diseases.

## Introduction

Commercial almond varieties are exclusively grafted on different rootstocks for a number of reasons. From the phytopathological point of view the most important of these reasons are the resistance of the rootstocks to various diseases, pests and nematodes as well as their tolerance to lime and to adverse conditions.

The most common rootstocks used for almonds are:

1. Almond seedlings of bitter almond which are well adapted to dry soils and exhibit some degree of resistance to soil borne fungal diseases, as well as seedlings of some commercial small-fruit variety.
2. Peach seedlings of the varieties: Lovell, Nemaguard (Nematode resistant), S 37 and S 60 resistant to lime and to nematode *Meloidogyne incognita* and GF 305.
3. Myrobalan plum and its hybrids, Mariana 2624 and 8/1.
4. Hybrids: almond x peach, peach x almond (GF 557 and GF 677) which are propagated by cuttings.
5. Crosses, almond x almond (Greek x Alnem N.<sup>o</sup> 1, 63 and 201) which are resistant to nematodes.

There are only a few data in the literature concerning the behaviour of almond and other stone fruit rootstocks to the main bacterial diseases of almond.

Almost all of them concern crown gall disease. Smith in 1925 tested 40 *Prunus* spp. for resistance to crown gall and found wide variations in susceptibility among species and among varieties of the same species. He found that peach (*P. persica*) seedlings were susceptible but other *Prunus* ssp. showed high resistance. Norton (1963) and his collaborators,

testing the behaviour of different peach rootstocks as Lovell, Nemaguard, S 37 and Rancho as well as different almond rootstocks to infections with *Agrobacterium tumefaciens*, found that there was a great variation in their susceptibility to crown gall among the different seedlings but in general all of them were susceptible. The peach rootstocks Harrow blood is considered as resistant to crown gall (LAYNE, 1974). Mitasov (1974) reported that 6 hybrid seedling rootstocks grafted with almond showed resistance to *Agrobacterium tumefaciens*. Resistance was also exhibited by myrobalan seedlings. He also reported that the scion had a substantial influence on disease incidence, so no infected trees were found on any of the rootstocks grafted with the variety Vynoslivil. The same influence of the scion on the crown gall incidence on the rootstocks GF-305 has also been reported (MARENAUD *et al.*, 1973). Popova (1972) reported that bitter almond rootstocks were more resistant than sweet almond ones the 1st year of growth, but all the healthy looking seedlings exhibited the disease the next year although they were transplanted to healthy soil. She concluded that probably the seedlings were latently infected with the pathogen.

## Crown gall

**Symptoms:** The main symptom of the disease is the formation of tumours (galls). The galls are usually formed at the crown or on the main roots (fig. 1).

In some cases galls can be formed on other parts of the plant (trunks or shoots). Galls vary in size, from nearly microscopic to 25 centimeters or more in diameter and their surface usually is smooth, when young, turning to rough when old. The effect the disease has on the tree (rootstock)

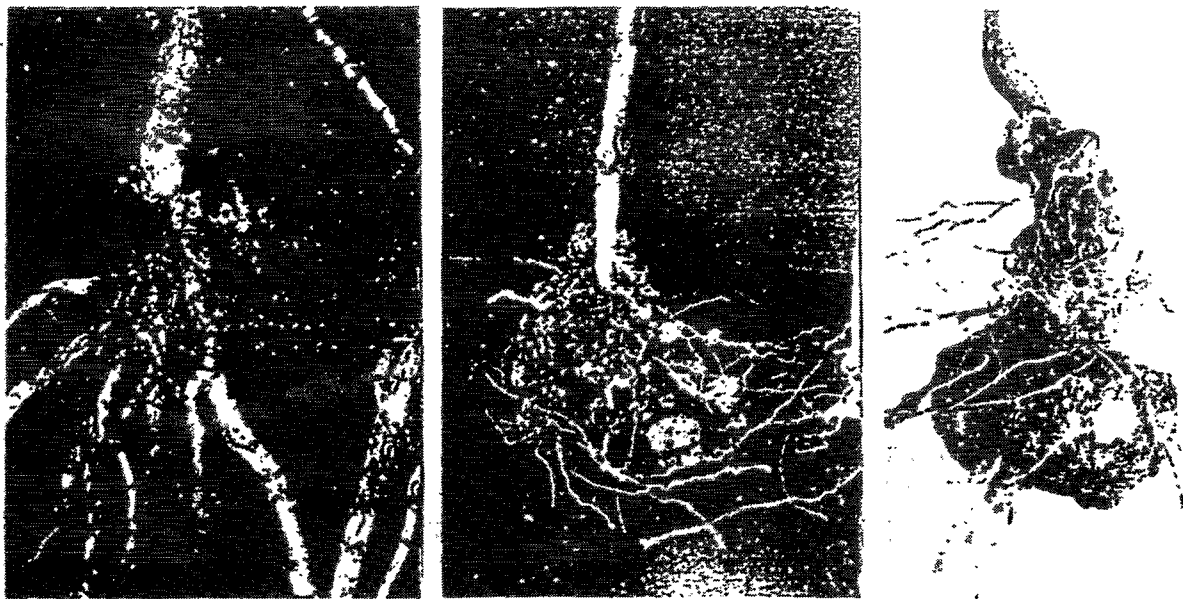


Fig. 1. Crown gall symptoms on GF-305 (a) and bitter almond (b, c) rootstocks.

depends on the location and number of infections, the size of the galls and whether secondary infections have occurred. The disease is more serious when infection occurs during the first 3 years of the plant life (ROSS et al., 1970). Galls on the crown area may cause serious damage by girdling action than galls located further out on the root system. The infected seedlings are less vigorous than the healthy ones, chlorotic and sometime die. Death may also occur through secondary fungal infection from the galls.

*The causal agent-epidemiology:* The disease is caused by the bacterium *Agrobacterium tumefaciens* which survives for long periods in the soil and has been detected in soils without disease history. The bacterium enters the plant tissues only through growth cracks and wounds, made either by the cultivation tools or by insects and nematodes (Orion and Zootra 1970). After the entrance and multiplication of the pathogen gall induction occurs. Most genes for virulence are located on a large (200 kb) plasmid (GARFINKEL and NESTER, 1984), the tumour-inducing (Ti) plasmid. Crown gall induction involves the transfer of part of this plasmid, the T-DNA of about 24 kb, from the bacterium to plant DNA (CHILTON et al., 1980). The process of induction is a complicated phenomenon and it seems that different compounds synthesized by the bacterium or the plant cells are involved.

It has been established (PANAGOPOULOS and PSALLIDAS, 1974; KERR and PANAGOPOULOS, 1977) that all pathogenic strains of *Agrobacterium tumefaciens* can be grouped on the bases of some biochemical and physiological characteristics, into three distinct biotypes (Table 1).

The biotype 1 and 2 strains of *Agrobacterium tumefaciens* have a wide host range and are responsible for the disease on

fruit trees and other hosts, while the strains of biotype 3 have a narrow host range and are responsible for the disease on grapevine (PANAGOPOULOS et al., 1978) and some other hosts. Biotype 3 strains have never been found causing crown gall on rootstocks of stone fruits and fruits trees in general.

Table 1  
DIAGNOSTIC CHARACTERS  
FOR DETERMINATION OF BIOTYPES  
OF *AGROBACTERIUM TUMEFACIENS*

	3-keto-lactose	2% NaCl	Max. growth temp.	Litmus milk	Acid from		Alkali from			Selective media of		
					erythritol	melezitose	malonate	L-tartrate	propionate	SCHROTH et al.	NEW and KERR	ROY and SASSER
Biotype 1	+	+	37	Alk. Redn.	-	+	-	-	+	+	-	-
Biotype 2	-	-	29	Acid.	+	-	+	+	-	-	+	-
Biotype 3	-	+	35	Alk.	-	-	+	+	-	-	-	+



### Crown gall control

Crown gall, as most of bacterial diseases, is difficult to control by chemical bactericides. Besides the usual reasons (lack of effective chemicals, difficulties in application, etc.) the nature of the disease (cancer) is also involved. The pathogen should be eliminated before disease initiation. Soil disinfection might seem the most effective measure but it is expensive, difficult to apply and sometimes not reliable, since treated soils are prone to recontamination and if it happens the severity of the disease is very high because of lack of competition from other soil organisms. Diseased nursery trees are usually discarded but because incipient infections may exist on healthy looking trees, all the sensitive nursery stock should be discarded. This accounts for the heavy losses caused by crown gall in stone fruit nurseries which were estimated to exceed the 6 million US dollars (ROSS et al., 1970).

### Biological control

KERR (1972) in Australia developed a biological control method for *Agrobacterium tumefaciens* based on the use of an antagonistic *Agrobacterium radiobacter* strain (K84) isolated from the rhizosphere of diseased plants. The control is achieved through a bacteriocin (Agrocin 84) produced by the antagonistic strain (KERR, 1980) which affects selectively the pathogenic strains.

The majority of the biotype 1 and 2 pathogenic strains of *A. tumefaciens* are sensitive to bacteriocin *in vitro* and are effectively controlled *in vivo*. There are a few pathogenic strains belonging to both biotypes which are resistant to the bacteriocin *in vitro* and consequently they are not controlled *in vivo*.

All the biotype 3 strains are resistant to agrocin 84. The method is simple to apply and highly successful under field conditions (KERR, 1972 and 1980; PSALLIDAS, 1988).

According to this method the seedlings, seeds or cuttings to be protected are dipped in a 10<sup>6</sup> cfu/ml suspension of the antagonistic bacterium just before planting or stratification. The antagonistic bacterium is provided either as fresh culture or as lyophilized preparation. There are also commercial products using peat impregnated with suspension of the antagonistic bacterium. The treated, this way, plants are protected against *A. tumefaciens* infections when planted in a contaminated soil.

Unfortunately there are some limitations in using the method of biological control.

First, as it was mentioned before, there are some strains which are insensitive to biocontrol, so before applying the method one should be sure that the pathogenic strains of *A. tumefaciens* prevailing in the area are sensitive to agrocin 84. In the Table 2 the reaction of 215 Greek isolates to agrocin 84 is presented.

Table 2

#### REACTION TO AGROCIN 84 AND POTENTIAL FOR BIOCONTROL OF GREEK ISOLATES OF *A. TUMEFACIENS*

No. of isolates	Biotype	Pathogenicity	Sensitivity to agrocin 84	Biocontrol potencial
42	1	+	+	+
30	1	—	—	+
72	2	+	+	+
3	2	+	—	—
17	2	—	—	—
51	3	+	—	—

+ = positive reaction, — = negative reaction

From this table it is obvious that all the biotype 1 Greek pathogenic isolates of *A. tumefaciens* are sensitive to biocontrol, all but 3 pathogenic isolates of biotype 2 are sensitive to biocontrol. The three insensitive strains were isolated from galls of Nemaguard rootstock from a single field, while none of the fifty one biotype 3 isolates was sensitive to biocontrol.

From the first experiments to investigate the effectiveness of biocontrol method using artificial inoculations of bitter almond seedlings it became apparent that the effectiveness of the method could be overcome by the appearance of new novel pathogenic forms, Table 3 (PANAGOPOULOS et al., 1978).

Table 3

#### AGROBACTERIUM FORMS FOUND IN GALLS ON PLANTS INOCULATED WITH A MIXTURE OF BIOTYPE 1 AND 2 PATHOGENS AND TREATED WITH *A. RADIOBACTER* STRAIN K84

Bacterial forms	Pathogenicity	Agrocin 84 production	Sensitivity to agrocin 84	Biotype
I	+	+	—	1
II	+	—	—	1.2
III	+	—	+	1.2
IV	—	—	+	1.2
V	—	—	—	1.2
VI	—	+	—	1.2 (K84)

+ = positive reaction, — = negative reaction

From these novel forms the form I of biotype 1 and form II of biotype 1 and 2 constitute a threat to biocontrol

since they are pathogenic and insensitive to agrocin 84. The biotype 1 of the VI form is a potential biocontrol agent since it is non pathogenic and produces the agrocin 84. The only plausible explanation of this phenomenon is that in the artificial inoculations, because of the inoculum force, tumors are initiated which favour the exchange of genetic material among the cells (ELLIS, 1979). The results indicate that K84 behaves as donor and biotype 1 cells as recipient because all biotype 2 novel strains have not acquired genes from K84 and *vice versa*.

It should be stressed that in field experiments with natural infected soils the effectiveness of the method was very high and from the spontaneous galls found in lateral roots, only sensitive strains were isolated.

### Resistant rootstocks

The use of resistant rootstocks in order to control crown gall has been recognized (SMITH, 1925) and many attempts have been made to find such rootstocks as we have already mentioned. Although some researchers claimed that some rootstocks exhibited resistance to crown gall such rootstocks have not been registered for almonds.

In the Pomology Institute of Naoussa and in the framework of the research programme concerning the study of the properties of different stone fruit rootstocks their behaviour to *Agrobacterium tumefaciens* infections was also investigated. The study started in 1977 and the results obtained are summarized in the following tables 4, 5, 6, 7 and 8.

Table 4

**BEHAVIOUR OF PEACH ROOTSTOCKS TO INFECTION BY *AGROBACTERIUM TUMEFACIENS* (MIXTURE OF BIOTYPE 1 AND 2) EXPERIMENT 1977-1978**

No	Rootstock	Disease severity (climax 0-5)					Remarks	
		0	1	2	3	4		5
1	IA 3	1	3	3	3	2	4	All healthy rootstocks next year exhibited the disease after reinoculation.
2	IA 30	2	4	1	2	0	11	
3	IA 11	5	1	4	0	0	3	
4	IA 22	0	1	1	3	4	8	
5	IA 27	6	2	2	2	2	1	
6	IA 20	3	4	4	4	2	5	
7	IA S37	2	13	5	3	1	1	
8	Nemaguard	0	1	3	3	2	1	

Table 5

**BEHAVIOUR OF PEACH AND ALMOND ROOTSTOCKS TO INFECTION BY *AGR. TUMEFACIENS* (BIOTYPE 1 AND 2). EXPERIMENT 1978-1989.**

No	Rootstock	Disease severity (climax 0-5)					
		0	1	2	3	4	5
1	IA 30	1	2	2	3	0	15
2	IA 3	3	6	2	0	0	13
3	IA 11	2*	1	7	5	2	10
4	IA 20	1*	4	6	8	5	12
5	IA 14	2	4	4	3	13	8
6	IA 32	2	3	4	3	4	11
7	IA 22	0	2	0	2	1	8
8	Nemaguard	10	6	4	5	3	2
9	GF 305	1	2	1	1	0	15
10	Marcona (Free pollination seeds)	0	7	2	4	0	0

\* Indicate seedlings which remained uninfected after the second inoculation.

Table 6

**BEHAVIOUR OF ROOTSTOCKS OF PEACH, ALMOND AND HYBRIDS TO INFECTION BY *AGROBACTERIUM TUMEFACIENS* (BIOTYPE 1 AND 2). EXPERIMENT 1979-80.**

No	Rootstock	Disease severity (climax 0-5)					
		0	1	2	3	4	5
1	IA 11	4	6	0	8	0	16
2	GF G77 (hybrid)	0	12	6	4	6	0
3	IA S37	0	2	8	0	0	18
4	IA 3	0	2	0	0	6	16
5	IA 20	2	0	6	6	0	20
6	GF 305	0	2	0	6	6	14
7	Nemaguard	4*	4	0	10	0	12
8	S37	0	0	0	0	7	14
9	IA Machrochori	0	2	0	0	0	14
10	IA 14	1	0	0	0	0	19
11	IA 2 (Kentrico)	0	0	0	0	0	20

\* From the 4 seedlings one remained uninfected after the second inoculation.

Table 7

**BEHAVIOUR OF PEACH, APRICOT AND ALMOND ROOTSTOCK SEEDLINGS TO INFECTIONS BY *AGROBACTERIUM TUMEFACIENS* (BIOTYPE 1 AND 2). EXPERIMENT 1980-82.**

No Rootstock	Disease severity (climax 0-5)					
	0	1	2	3	4	5
1 Stella	13*	0	0	0	0	0
2 Early Orange	7	0	0	0	0	0
3 IΔ 20	0	4	16	2	0	0
4 IΔ 32	0	3	6	6	3	0
5 IΔ 3	0	3	7	4	0	0
6 Nemaguard	0	0	0	0	20	0
7 Ferragnes X Italian var.	0	10	8	12	0	2 Almond
8 10/20/67 X Ferragnes	1	5	10	10	0	0 "
9 Xirolimni X Italian var.	0	5	15	0	0	0 "
10 IΔ 20 reinoculation	1	1	0	0	0	0
11 IΔ 11 reinoculation	1	1	0	0	0	0
12 Nemegard reinoculation	0	1	0	3	0	0

\* Three seedlings remained healthy after reinoculation.

Table 8

**BEHAVIOUR OF PEACH, APRICOT, ALMOND AND HYBRIDS ROOTSTOCK SEEDLINGS AND ROOTED CUTTINGS TO THE INFECTION BY CROWN GALL *AGROBACTERIUM TUMEFACIENS* (BIOTYPE 1 AND 2). EXPERIMENT 1982-83.**

No Rootstock	Disease severity (climax 0-5)					
	0	1	2	3	4	5
1 Red Leaf	2	0	0	5	0	14
2 Siberian C	3	5	0	7	0	27
3 Nemaguard	7	2	1	1	1	8
4 IΔ 22	0	0	0	0	0	14
5 Amygd. webbii	13	1	0	2	0	0
6 G F 305	0	1	0	0	0	24
7 IΔ 3	0	1	0	0	0	8
8 IΔ 20	2	6	0	8	0	48
9 GF 677 (hybrid)	0	0	9	0	0	24
10 IΔ 20 3rd inoculation	0	1	0	0	0	0
11 IΔ 11 3rd inoculation	0	1	0	0	0	0
12 Nemaguard 3rd inoculation	0	1	0	0	0	0
13 Stella 2nd inoculation	0	1	2	4	0	0
14 Early orange 2nd inoculation	0	1	2	4	0	0
15 Cuttings IΔ 20	8*	0	0	0	0	0
16 Cuttings IΔ 11	6*	0	0	0	0	0

\* The cuttings were taken from the selected as resistant after 2 reinoculations.

These results are in agreement with those of other investigators, as has already been described in the introduction. There is a great diversity in the resistance that seedlings of different species and varieties of stone fruits exhibit at the 1st year, but in subsequent inoculations they behave differently. The three seedlings (one IΔ 20 and two IΔ 11) which are considered as resistant because the cuttings were not infected, were planted in big pots and kept in the screen house for further investigation.

### Hyperplastic bacterial canker

#### Symptoms

The disease was described by Psallidas and Panagopoulos in 1968 in the island of Crete, Greece.

The characteristic symptom of the disease is the formation of swollen cankers on branches, twigs, shoots and trunks (fig. 2).

Usually the cankers begin from the leaf scars but any wound can serve as an entrance for the pathogen. The first symptoms appear in late winter as the dormancy breaks, as a swelling of the bark in the place around the infected leaf scar or wound. Later the affected bark tissues split apart and open cankers are formed. The cankers are surrounded by swollen rough dark brown margins. These cankers are perennial being active not only throughout the year but also for many years. The sire of the canker depends on its age, thus the length of cankers on two year old shoots is between 3 and 5 cm. while in older branches and trunks cankers 15-20 cm. long may be found.

#### Causal agent-epidemiology

The disease is caused by the bacterium *Pseudomonas amygdali* (PSALLIDAS and PANAGOPOULOS, 1975).

The bacterium overwinters inside the infected plant tissues and is disseminated in long distances by infected propagating material. The pathogen does not have apiphytic life.

The pathogenic bacterium is host specific infecting only almond (*Prunus dulcis*). Seedlings of bitter almond were very sensitive to *P. amygdali* (PSALLIDAS, unpublished data). Other *Prunus* species were not infected by the bacterium in artificial inoculations. Some resistance has been found in certain commercial almond varieties (PSALLIDAS and STYLIANIDES, 1985).



Fig. 2. Hyperplastic bacterial canker symptoms (a) infected almond tree (b) girdled branch.

## Almond leaf scorch, peach phony, plum leaf scald diseases

### Symptoms

#### 1. ALMOND SCORCHING

The most characteristic symptom of the disease (MOLLER et al., 1974) is leaf scorching which starts as marginal chlorosis and extends to the whole leaf. Leaf scorch is followed by decrease productivity, general decline and subsequent death of the tress. The first symptoms appear on the affected trees about mid-june. The scorched leaves remain on the trees until fall defoliation.

#### 2. PEACH PHONY

The infected trees show severe dwarfing, of new growth and fruits. As a result smaller crops and undersized fruits are produced. The trees are more compact and flattened than the normal trees and the leaves tend to be greener. Phony disease does not induce early death of affected trees.

#### 3. PLUM LEAF SCALD

The first symptom is a light chlorosis or browning at the tips or the margins of the leaves. The chlorotic areas later become brown and dry, and are separated from the unaffected areas by light yellow margin. As the disease progresses several necrotic bands on leaves usually appear and later the whole plant is affected and fruit size quality and yield are reduced (RAJU et al., 1982).

### The causal agent-epidemiology

All the above diseases are caused by the same bacterium (WELLS et al., 1987) which is a fastidious xylem limited bacterium. It has a wide host range infecting many plants belonging to different genera. The same bacterium is responsible for the Pierce's disease of grapevine.

The bacterium is transmitted by several species of leafhoppers.

It is also transmitted with infected propagation material, both rootstocks and scions, and spreads upwards and downwards respectively.

### Control

Control measures should include careful selection of propagation material to avoid affected trees, and vector control to restrict the spread of the disease from infected plants in the vicinity of the orchards.

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