agronomie (1996) 16, 247-256
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Behaviour of *Prunus* cultivars and hybrids towards *Agrobacterium tumefaciens* estimated from hardwood cuttings

A Pierronnet*, G Salesses

Unité de recherches sur les espèces fruitières et la vigne, Centre de Bordeaux, Inra, BP 81, F-33883 Villenave-d'Ornon cedex, France

(Received 5 July 1995; accepted 29 March 1996)

Summary — Using the hardwood cuttings method, the behaviour of different *Prunus* spp, which can be used as rootstocks, was evaluated for susceptibility to the telluric bacteria *Agrobacterium tumefaciens* that induces crown gall. The results show that: i) for *Prunus cerasifera* all the clones studied are susceptible (among them the clone P2032 seems the least susceptible), and so are the different hybrids issued from crosses including *P cerasifera*, except P2038 which is an hybrid between *P besseyi* and *P cerasifera*; ii) for *Prunus domestica* there is a low level of susceptibility among the clones and the hybrids issued from crosses including *P domestica. P domestica* seems interesting to use in breeding programs to introduce a low susceptibility to *A tumefaciens*.

***Agrobacterium tumefaciens* / crown gall / *Prunus* / rootstock / susceptibility**

Résumé — Comportement d'hybrides et de cultivars de *Prunus*, vis-à-vis d'*Agrobacterium tumefaciens*, estimé à partir de boutures ligneuses. *En utilisant la méthode de boutures ligneuses, le comportement de différents* Prunus, *pouvant servir de porte-greffe, est étudié vis-à-vis de la bactérie tellurique* Agrobacterium tumefaciens, *microorganisme responsable de la galle du collet. Les résultats montrent que : i) pour les* Prunus cerasifera, *tous les clones étudiés sont sensibles (parmi eux, le clone P2032 semble le moins sensible) ainsi que les différents hybrides issus de croisements comportant* P cerasifera, *excepté le P2038 qui est un hybride entre* P besseyi *et* P cerasifera *; ii) pour les* Prunus domestica, *le niveau de sensibilité est faible tant parmi les clones que parmi les hybrides ayant* P domestica *comme parent.* P domestica *apparaît intéressant à utiliser dans des plans de croisements, pour introduire une moindre sensibilité à* A tumefaciens.

Agrobacterium tumefaciens / *galle du collet* / Prunus / *porte-greffe* / *sensibilité*

INTRODUCTION

Crown gall is a tumoric disease caused by a motile Gram-negative telluric *Eubacterium*: *Agrobacterium radiobacter*, Beijerinck and Van Delden, Conn pv *tumefaciens*, Smith and Towsend (Kesters and De Ley, 1984) usually called *Agrobacterium tumefaciens*. Among the dicotyledons, a large number of species are susceptible, particularly in the *Prunus* genus (Faivre-Amiot, 1982). Generally, the monocotyledons do not appear to be susceptible to *A tumefaciens*

* Correspondence and reprints

(Gautheret, 1980), though some reports mentioned the *Agrobacterium*-mediated cell transformation in some species of this class (Douglas et al, 1985; Hernalsteens et al, 1984; Hooykaas-Van Slogteren et al, 1984).

A tumefaciens is attracted to the exudates of wounded plant tissues (Shaw et al, 1988). It becomes pathogenic following attachment to the injured plant cells (Matthysse et al, 1981) and transfer of its Ti-plasmid T-DNA to the plant cell nuclear genome (Chilton et al, 1977). The T-DNA of virulent strains has an oncogenic region coding for products that upset the normal regulation of phytohormone synthesis in the plant cell and induce tumour growth by proliferation of the transformed cells (Tempé and Shell, 1987; Armitage et al, 1988). Large tumours generally weaken and may occasionally kill the plant (Faivre-Amiot, 1982).

Chemical control of this bacterium is inefficient, and disease prevention using K84 or K1026 strains of *A radiobacter* var *radiobacter* are only efficient against susceptible strains of *A tumefaciens* (Kerr and Panagopoulos, 1977; Jones and Kerr, 1989; Ryder and Jones, 1991).

Therefore the identification of plant genetic variability for resistance to *A tumefaciens* and its subsequent exploitation in breeding programmes, would be of significant agronomic value. This approach to the control of *A tumefaciens* has been adopted previously for poplar (Nesme et al, 1990; Beneddra, 1990) and the first results, obtained with the hardwood cuttings method, that already had given a good estimation of the susceptibility of some different *Prunus* rootstocks (Pierronnet and Escalettes, 1990; Pierronnet and Eyquard, 1993) were encouraging enough to undertake this study with a larger number of different *Prunus*.

In the present study the objective was to identify possible sources of genetic resistance to *A tumefaciens* in *Prunus* spp and their interspecific hybrids which could be of value in the rootstock breeding programme at the Unité de recherches sur les espèces fruitières et la vigne de Bordeaux. A first approach of the genetic determinism of resistance is undertaken.

MATERIALS AND METHODS

Plant material

The plant material consists of 87 *Prunus* of different genetic origins, representing eleven species as clones or as intraspecific or interspecific hybrids (table I). Its ploidy level varies from diploid ($2n = 16$) to hexaploid ($2n = 48$). Two hybrids issued from Damas de Toulouse are aneuploid because of the production, by the pentaploid Damas, of gametes with chromosomes number varying from 16 to 24. According to Salesses (1977) Damas de Toulouse is an interspecific hybrid: *P domestica* (or *P insititia*) x *P spinosa*. The different ploidy levels of the Marianna plum descendants, P8-1 (triploid), P8-13 and P10-2 (both pentaploid) could be explained, first by interspecific hybridization, second by the production of polyploid gametes by the diploid Marianna plum. So, P8-1 is probably issued from a cross between Marianna plum and *P cerasifera* (Salesses, 1977) whereas P10-2 and P8-13 could originate from crosses between Marianna plum and a hexaploid plum *P domestica* or *P insititia* (Bernhard, personal communication).

This material is under evaluation or already used as rootstock (table VI).

Bacterial material

Strain AT 78A025 of *A tumefaciens* was isolated from infected *Prunus persica* L by Faivre-Amiot (personal communication). It is a nopaline strain which has a well established aggressiveness towards the *Prunus* genus.

Bacteria were cultured on a potato medium containing 20 g of sucrose, 20 g of agar-agar, 5 g of NaCl, 500 mL of water in which 200 g of peeled potatoes were boiled for 20 minutes, and 500 mL of distilled water. Bacterial cultures were incubated at 20 °C for four days and then used for the inoculation of *Prunus* cuttings at a concentration $>10^8$ cfu/mL.

Auxin solution

A solution of indolbutyric acid (IBA) was prepared by dissolving 200 mg of IBA in 30 mL of ethanol and then diluting to a final volume of 100 mL with distilled water (ie, 2 000 µg IBA/mL).

Inoculation of plant material

Hardwood cuttings of 15-cm length were harvested from the orchard following leaf fall (November). Sixty cuttings were selected for each clone, progeny or hybrid, and were stored in polyethylene bags for about one month in a cold room (4 °C at 100% relative humidity).

At the beginning of December, after recutting the basal end to make a fresh wound, the cuttings were inoculated by dipping the base in the bacterial suspension or in auxin solution for the control. The IBA treated cuttings were only used as a control of the plant material viability during the experiment. For each treatment, 30 cuttings were used.

Table I. Origin, clone number, and chromosome number of the 87 evaluated Prunus.

Genetic origin	*Number of clone or hybrid*	*2n*
P besseyi	P3495	16
P besseyi x *P cerasifera*	P2037	16
P besseyi x *P cerasifera*	P2038	16
P besseyi x *P persica*	P3400	16
P cerasifera	P18	16
P cerasifera	P1079	16
P cerasifera	P1090	16
P cerasifera	P1254	16
P cerasifera	P2032	16
P cerasifera	P2175	16
P cerasifera	P2980	16
P cerasifera x *P cerasifera*	P(2175x2032) (9 hybrids)	16
P cerasifera x *P cerasifera*	P(2032x2175) (10 hybrids)	16
P cerasifera x *P cerasifera*	P(2175x1079) (16 hybrids)	16
P cerasifera x *P cerasifera*	P(2032x1079) (15 hybrids)	16
P cerasifera x (*P cerasifera* x Japanese plum)	P[1079x(16-5x2128)2]21	16
P cerasifera x Japanese plum	P(16-5x2128)2	16
Damas de Toulouse	P1869	40
Damas de Toulouse self pollination	P1869-4	41
Damas de Toulouse x P domestica	P(1869x328)48	45
P dasycarpa	P2315	16
P domestica x *P cerasifera*	P(2036x1079)2	32
P domestica x *P cerasifera*	P(2036x16-5)3	32
P domestica x *P domestica*	P3116	48
P domestica x *P spinosa*	P(253x106)77	40
P domestica x *P spinosa*	P(707x55)16	40
P domestica x Japanese plum	P(2036x2069)2	32
P insititia x *P domestica*	P3295	48
P insititia (Saint-Julien-d'Orléans) progeny	St Julien 58-7	40
P insititia (Saint-Julien-d'Orléans) progeny	St Julien GF 655-2	48
Japanese plum x (*P cerasifera* x Japanese plum)	P[2128x(16-5x2128)2]16	16
Japanese plum x (*P cerasifera* x *P persica*)	P3293	16
Japanese plum x *P persica*	P3294	16
Japanese plum x *P spinosa*	P3297	32
Marianna plum	P2736	16
Marianna plum progeny	P8-1	24
Marianna plum progeny	P8-13	40
Marianna plum progeny	P10-2	40
P persica x *P davidiana*	P3296 (P41-4-21)	16
P spinosa x *P cerasifera*	P(106x2175)5	24
P spinosa x *P domestica*	P(33-0x1249)17	40

p = Plum or prune; S = peach; SJ = Saint-Julien-d'Orléans; Sp = *Prunus spinosa*; progeny = obtained from open pollination; P18 = clone of Myrobalan B; P213 = cv Pershore; P253 = cv Reine-Claude d'Althan; P322 = cv Belsiana (*P cerasifera* x *P triflora*) (Bernhard, 1962); P328 = cv Mme Guttin; P707 = clone of French prune (Prune d'Ente); P871 = *P cerasifera* x *P persica*; P1249 = cv Krasinsky; P1869 = Damas de Toulouse [P domestica (or *P insititia*) x *P spinosa*] (Salesses, 1977); P2036 = cv Stanley; P2069 = cv Methley (*P cerasifera* x *P salicina*) (Day and Tufts, 1944); P2128 = cv Burbank (seedling imported from Japan by Burbank); P2736 = Marianna plum (issued from *P cerasifera* and *P munsoniana*, Salesses, 1977); P3116 = Reine-Claude vraie P994 x Reine-Claude d'Althan P148; P3293 = P322 x P871; P3294 = P322 x S1058; P3295 = SJ53 x P213; P3296 = *P persica* x *P davidiana* (cv Avimag-Cadaman); P3297 = P2069 x Sp106; S 1058 = cv Yunnan.

After drying, the cuttings were packed in polyethylene bags and stored for 20 days at room temperature (approximately 20 °C) in the dark to initiate either gall (treated cuttings) or callus (control cuttings) formation.

All cuttings were then put in containers under favourable conditions for rooting, ie, with moderate watering, in a cool greenhouse with the basal ends placed into a gravel/sand mixture (50:50).

Calculation

After three months, the diameter (*D*) of the galls induced by *A tumefaciens* and the diameter (*d*) of the cuttings just above the gall were measured. The difference was then calculated for each cutting as $\Delta = D - d$ and the mean 'Δ' of 30 replicates calculated.

An analysis of variance was carried out on the data using Amance software (INRA, Centre de Nancy) and the Bonferroni test was used to calculate significant differences between the clones or hybrids responses to *A tumefaciens* infection.

As there is no reference material for behaviour vis-à-vis this extant disease and in order to give some insight on the ranking of the various genotypes in this study, we have used Bonferroni's multiple comparison test following an analysis of variance in order to tentatively distinguish groups, in spite of the shortcomings of such a procedure.

RESULTS

P cerasifera *clones and their hybrids*

P2032, P1079 and 15 hybrids obtained from these two genotypes (P2032 used as female) were evaluated for their behaviour towards *A tumefaciens*. The results, summarized in table II, show that P2032 is significantly less susceptible than P1079 and their hybrids. Most of the hybrids P(2032 x 1079) are as susceptible as P1079 and three hybrids (16 - 38 - 14) are more susceptible.

P1079 was also used as male parent in a cross with the *P cerasifera* clone P2175. The two parents and 16 hybrids, were evaluated for their behaviour towards *A tumefaciens* (table III).

As in the previous cross we can observe a high level of susceptibility.

The results show that there is no significant difference between P2175, P1079 and ten of their hybrids whereas six and three hybrids are respectively more susceptible than P2175 and than P1079.

If we compare the susceptibility of these two series of hybrids obtained using P1079 as male parent (fig 1), we can observe a predominant

Table II. Response to *A tumefaciens* of two *Prunus cerasifera* clones, P2032 and P1079, and their hybrids.

Clone or hybrid no	*'Δ' (mm)*	*Groups of 'Δ'*
P2032	**3.17**	a
P(2032x1079)23	5.36	b
P(2032x1079)21	5.90	c
P(2032x1079)3	5.95	
P(2032x1079)6	6.14	d
P1079	**6.41**	
P(2032x1079)36	6.56	
P(2032x1079)33	6.57	
P(2032x1079)24	6.70	e
P(2032x1079)18	6.89	
P(2032x1079)32	7.00	f
P(2032x1079)12	7.05	
P(2032x1079)13	7.26	
P(2032x1079)27	7.33	
P(2032x1079)16	7.56	
P(2032x1079)38	8.10	
P(2032x1079)14	8.26	

The statistically significant difference of the groups was established at the probability level $p = 0.05$.

Table III. Response to *A tumefaciens* of two *Prunus cerasifera* clones, P2175 and P1079, and of their hybrids.

Clone or hybrid no	*'Δ' (mm)*	*Groups of 'Δ'*
P2175	**5.60**	a
P(2175x1079)29	5.66	b
P(2175x1079)36	5.76	
P(2175x1079)15	5.83	
P(2175x1079)2	5.96	
P(2175x1079)31	6.07	
P1079	**6.41**	c
P(2175x1079)16	6.56	d
P(2175x1079)38	6.58	
P(2175x1079)39	6.60	
P(2175x1079)14	6.77	e
P(2175x1079)11	6.85	
P(2175x1079)3	6.95	
P(2175x1079)6	7.47	
P(2175x1079)37	7.74	
P(2175x1079)19	7.78	
P(2175x1079)12	8.00	
P(2175x1079)22	8.02	

The statistically significant difference of the groups was established at the probability level $p = 0.05$.

effect of the most susceptible parent P1079. The hybrids are grouped around P1079 and no hybrid presents the same low level of susceptibility as P2032.

In the case of P2032 and P2175, reciprocal hybrids were available. Nine P(2175 x 2032) hybrids and ten P(2032 x 2175) hybrids were simultaneously tested comparatively to their parents (table IV).

Among the hybrids issued from the clone P2032, only one, (–12), issued from P2032 used as male parent, shows the same behaviour as P2032, while the others are significantly more susceptible. This hybrid (–12) is significantly less susceptible than P2175. Among the others, fifteen hybrids, (–49) to (–24), are as susceptible as P2175 and three are more susceptible; among the former, eight have P2032 as male parent and seven as female parent. It is worth noting that the most susceptible group of hybrids (group h) is composed exclusively of six hybrids issued from P2032 used as female parent.

The comparison between the overall mean ['Δ'] of the P(2175 x 2032) hybrids and that of the P(2032 x 2175) hybrids shows a significant difference at $p = 0.02$. The P(2175 x 2032) family, with ['Δ'] = 5.35, is significantly less susceptible than the P(2032 x 2175) family with ['Δ'] = 6.29.

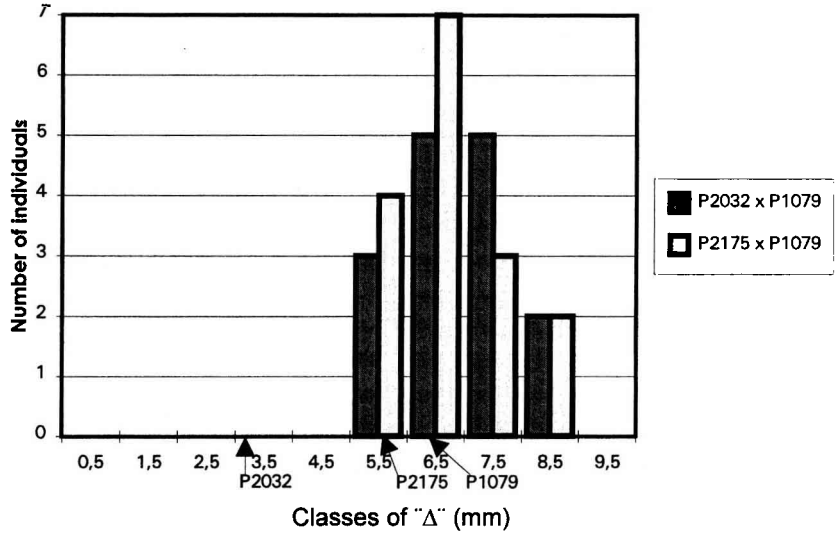

Fig 1. Distribution of the hybrids P(2175 x 1079) and P(2032 x 1079) for 'Δ'.

Table IV. Response to *A tumefaciens* of two *Prunus cerasifera* clones, P2032 and P2175, and their hybrids from reciprocal crosses.

Clone or hybrid no	*'Δ' (mm)*	*Groups of 'Δ'*
P2032	**3.17**	a
P(2175x2032)12	3.65	b
P(2032x2175)49	4.72	c
P(2175x2032)7	4.80	d
P(2032x2175)11	5.19	e
P(2175x2032)9	5.21	
P(2175x2032)27	5.31	f
P2175	**5.60**	
P(2175x2032)1	5.66	
P(2175x2032)25	5.69	g
P(2175x2032)33	5.83	
P(2032x2175)27	5.88	
P(2175x2032)30	5.93	
P(2032x2175)52	6.04	
P(2175x2032)16	6.08	
P(2032x2175)54	6.24	h
P(2032x2175)47	6.36	
P(2032x2175)24	6.71	
P(2032x2175)38	7.11	
P(2032x2175)4	7.13	
P(2032x2175)10	7.58	

The statistically significant difference of the groups was established at the probability level $p = 0.05$.

The distribution for 'Δ' of reciprocal hybrids and their parents (fig 2) shows a difference in susceptibility between the hybrids issued from both crosses according to the direction of the cross between the two parents.

Prunus spp and interspecific hybrids

The behaviour of eleven *Prunus* species, and different interspecific hybrids, is presented in table V and illustrated in figure 3.

Table V and figure 3 show that there is a large variability of the behaviour according to genetic origin (classes from 0.5 to 7.5). We can consider that there are two levels of susceptibility in the tested *Prunus* estimated by the 'Δ' value. The 'Δ' range 0–3, corresponds to the least susceptible, while the 'Δ' ranges 3–8 groups the susceptible clones or hybrids.

The data analysis of table V shows that *P domestica* (D) and its progenies display interesting behaviour and are among the least susceptible: 'Δ' < 1.5 and 'Δ' < 3 for five and nine,

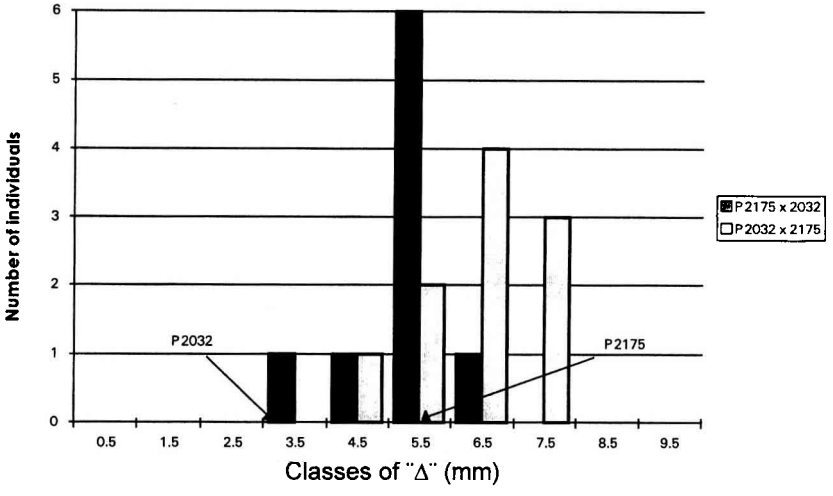

Fig 2. Distribution of the hybrids compared with P2175 and P2032 for 'Δ'.

respectively, out of 11 individuals having *P domestica* as parent. Most of the hybrids issued from *P domestica* show a rather good behaviour and the two hybrids with a *P domestica* as a parent and giving a value of 'Δ' > 4.5, ie, P(2036 x 1079)2 and P(2036 x 16-5)3, have *P cerasifera,* P1079 and P16-5 respectively, both showing the same level of susceptibility (Pierronnet, unpublished data) as second parent.

The majority of *P cerasifera* clones and their progeny have a value of 'Δ' > 3 and the clones P2980 and P2032 appear as the less susceptible (respectively 'Δ' = 2.74 and 3.17) among the tested individuals of this species.

P spinosa and the Japanese plum, used in this work, seem to have no significant effect on the hybrid susceptibility when hybridized with either *P domestica* (moderately susceptible) or *P cerasifera* (relatively susceptible).

P3495, the only clone of *P besseyi* evaluated, is among the least susceptible ('Δ' = 2.50). Two hybrids issued from a natural cross between a same *P besseyi* and *P cerasifera* were evaluat-

Table V. Behaviour of different species and hybrids of Prunus.

Genetic origin	*Clone no*		*'Δ' (mm)*	*Groups of 'Δ'*
IxD	P3295	R	0.59	a
BxC	P2038	R	0.67	b
DaxD	P(1869x328)48		1.12	c
PxDv	P3296	R	1.17	d
D	P3116	R	1.24	e
DxSp	P(253x106)77		1.32	f
DxJa	P(2036x2069)2		1.39	g
BxP	P3400		1.45	h
M prog	P8-13		2.05	i
JaxP	P3294	R	2.18	j
DxSp	P(707x55)16		2.21	k
M prog	P10-2		2.29	l
B	P3495		2.50	m
C	P2980		2.74	n
MxC	P8-1	R	2.84	o
SpxD	P(33-0x1249)17		2.89	p
C	P2032	R	3.17	
Ja(CxJa)	P[2128x(16-5x2128)2]16		3.18	
I prog	GF 655-2	R	4.15	
BxC	P2037		4.16	
DxC	P(2036x1079)2		4.51	
DxC	P(2036x16-5)3		4.62	
I prog	SJ 58-7	R	4.68	
Ja(CxP)	P3293	R	4.70	
Da	P1869-4		4.84	
Dy	P2315		5.17	
C	P1090	R	5.29	
C	P18	R	5.35	
CxJa	P(16-5x2128)2		5.51	
C	P2175		5.60	
Da	P1869		5.78	
C	P1254		5.88	
C	P1079		6.41	
SpxC	P(106x2175)5		7.11	
JaxSp	P3297	R	7.27	
M	P2736	R	7.93	

The statistically significant difference of the groups was established at the probability level $p = 0.05$. List of abbreviations for the genetic origin. B = *P besseyi*; C = *P cerasifera*; D = *P domestica*; Da = Damas; Dv = *P davidiana*; Dy = *P dasycarpa*; I = *P insititia*; Ja = Japanese plum; M = Marianna plum; p = *P persica*; Sp = *P spinosa*. R = already used as rootstocks; prog = seed obtained from open pollination.

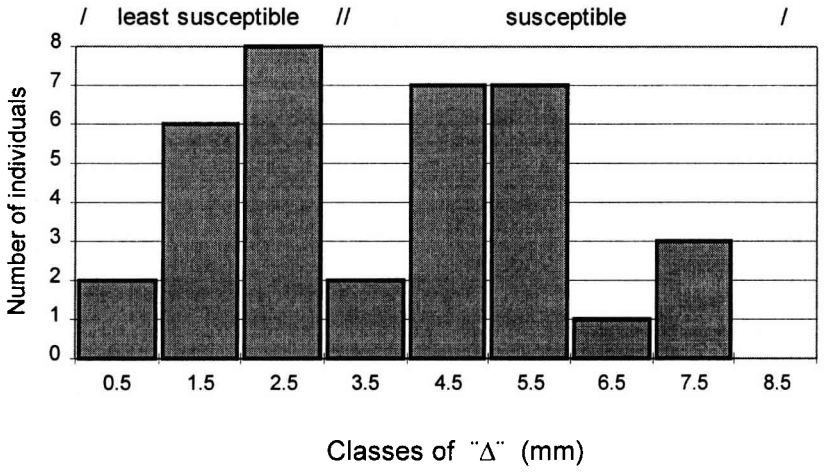

Fig 3. Distribution for 'Δ' of all the *Prunus*, clones or hybrids, evaluated towards *A tumefaciens*.

ed: one, P2038, shows a good behaviour ('Δ' = 0.67), whereas the second appears susceptible ('Δ' = 4.15).

The Marianna plum clone P2736 is very susceptible but we observe the low susceptibility of P8-13 and P10-2, both pentaploid hybrids obtained from open pollination of Marianna plum, likely by an hexaploid *Prunus*, *P domestica* or *P insititia*; (P8 and P10 are two numbers of clone from the same mother-tree of Marianna plum, Bernhard, personal communication).

Damas de Toulouse P1869, P1869-4 (issued from a self pollination of P1869), and the clone P2315 of *P dasycarpa* which is originating from a cross between *P armeniaca* and *P cerasifera* (Rehder, 1940), are susceptible.

In this study the behaviour of 13 rootstock clones, selected at INRA and registered into the French official Catalogue des espèces et variétés, was investigated (table VI). These rootstocks were identified as moderately susceptible (five clones) or susceptible (eight clones).

The results for Avimag (clone P3296) shown in table V are not valid due to excessive gall necrosis caused by prolonged contact with overmoist sand. In previous experiments, P3296 was found to be susceptible (Pierronnet, unpublished data).

DISCUSSION

The entire wounding of the cambium ring, the use of a very concentrated bacterial suspension

Table VI. Response to *A tumefaciens* of the 13 registered Prunus rootstocks.

Clone name	*Clone no*	*Trademark*	*Genetic origin*	*Behaviour*
Ferdor	P3295	Julior®	*P insititia* x *P domestica*	mod suscep
Ferlenain	P2038	Plumina®	*P besseyi* x *P cerasifera*	mod suscep
Avifel	P3116	Torinel®	*P domestica* x *P domestica*	mod suscep
Yumir	P3294	Myran®	Japanese plum x *P persica*	mod suscep
GF 8-1	P8-1		Marianna plum x *P cerasifera*	mod suscep
Myrest	P2032	Myrabi®	*P cerasifera*	susceptible
Saint-Julien	GF 655-2		*P insititia* progeny	susceptible
Fercien	SJ 58-7		*P insititia* progeny	susceptible
Ferciana	P3293	Isthara®	Japanese plumx(*P cerasifera* x *P persica*)	susceptible
Fercino	P1090	Myrocal®	*P cerasifera*	susceptible
GF 1869	P1869		Damas de Toulouse	susceptible
Fereley	P3297	Jaspi®	Japanese plum x *P spinosa*	susceptible
Avimag	P3296	Cadaman®	*P persica* x *P davidiana*	susceptible

mod suscep = moderately susceptible.

for inoculation, and the calculation of 'Δ' issued from 30 cuttings are factors which minimize the risk of having, like Cardenas (1975), difficulty in interpretation of the results due to gall size variation. The control of cultural conditions in the greenhouse allows the obtention of more homogeneous results than those obtained outdoor, by us (Pierronnet, unpublished data) or by Nesme et al (1990) on poplar.

The results obtained confirm numerous observations made under orchard or nursery conditions, as well as data published by Smith (1925) and those obtained more recently by Pierronnet and Escalettes (1990) and Pierronnet and Eyquard (1993). The relatively high and low susceptibilities of *P cerasifera* and *P domestica* respectively were already observed in 1925, although Smith mentioned some variability within the *P domestica* group.

Within the *P cerasifera* species, P2980 and P2032 are the least susceptible clones. For the hybrids used in this experiment, raised from P2032, no inheritance of this low susceptibility can be seen. On the contrary, there is a higher susceptibility in all the hybrids compared to P2032 (except the -12), and for 13 out of 19 hybrids compared to P2175, particularly for the hybrids P2032 x P2175. It is worth noting that, in hybrids involving P2032 and P2175, P2032 confers a greater susceptibility when used as female parent. The existence of a maternal effect exerted by P2032 for higher susceptibility remains to be confirmed. To test the validity of this observation, further work using a larger number of hybrids is necessary.

In interspecific crosses, the few hybrids of *P cerasifera* used in combination with either *P spinosa*, Japanese plums or *P domestica* cv Stanley clone P2036 are susceptible. On the other hand, the results for *P domestica* show that for this species there is a tendency towards a low susceptibility. This characteristic could be transmitted to the few hybrids tested, issued from crosses between *P domestica* and other species such as *P spinosa*, Marianna plum or the Japanese plums.

In the case of crosses between *P domestica* cv Stanley, previously evaluated and found moderately susceptible, and *P cerasifera* P1079 or P16-5, the hybrids obtained have a susceptibility which is intermediate to that of the parents. P16-5 has been found to be as susceptible as P1079 in a previous investigation (Pierronnet, unpublished data).

The behaviour of the tested clone of *P besseyi* is equivalent to that of the least susceptible *P cerasifera* (P2032 and P2980) and the susceptibilities of two sibling hybrids issued from the cross of one *P besseyi* with *P cerasifera* are very different: P2037 is susceptible and P2038 may be considered as resistant. We can only note the good behaviour of P2038, confirming a previous observation (Pierronnet and Escalettes, 1990).

The experimental conditions, previously described for investigating susceptibility to *A tumefaciens,* require specific conditions necessary to get reliable responses. Particular attention should be paid to temperature management and to sand moisture level since frost or root asphyxia can easily cause necrosis of the tissues developing during the period of gall swelling.

The use of only one strain of *A tumefaciens* may be criticized. In a first step only the strain AT 78A025, which was previously isolated from the experimental site, and evaluated in numerous occasions as very aggressive, was chosen . The introduction of other potentially aggressive strains of *Agrobacterium* into the experiments must be taken into account but at this time their utilization in the experimental site for in vivo experiments was considered undesirable.

With the exception of two clones (P1090, P1869), the results obtained from this in vivo experiment were in agreement with those obtained from an in vitro experiment (Pierronnet and Escalettes,1990) for the appreciation of the susceptibility. This agreement needs to be re-examined since in vitro experimentation offers the advantage of working with several aggressive bacterial strains.

CONCLUSION

Our previous observations, carried out in orchard or nursery, have pointed out the susceptibility of different *Prunus* rootstocks to *A radiobacter* pv *tumefaciens.*

The present study, using the previously described cuttings method as an easy early test of selection, indicates tendency to susceptibility for *Prunus cerasifera* and to low susceptibility for *Prunus domestica.* It is possible to use genitors belonging to this species in a breeding programme which includes crown gall resistance as one of the selection criteria, in order to get rootstocks presenting a good low level of susceptibility to this pest.

ACKNOWLEDGMENTS

We thank JP Eyquard for his technical collaboration and A Bonnet for his assistance in the greenhouse.

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