Correlation Between Susceptibility to Crown Gall and Sensitivity to Cytokinin in Aspen Cultivars

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ABSTRACT

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Closely related aspen cultivars (*Populus tremula* × *P. alba*) were ranked as resistant, intermediate, or susceptible to crown gall according to their tumor response to the selected strains C58, B6, and 354 of *Agrobacterium tumefaciens*, respectively. As shown with mutant agrobacteria that harbored derivatives of pTiB6S3, a strain retained its ability to define differences in susceptibility among aspen cultivars as long as it had a functional *ipt* gene and, therefore, could induce the biosynthesis of a cytokinin in transformed plants. This suggested that differences in susceptibility to crown gall were related to differences in sensitivity to cytokinin. Cultivar sensitivity to cytokinin was determined in vitro by a leaf disk assay. Aspen cultivars resistant to the highest cytokinin concentrations (32 μM benzyladenine) were also found to be resistant to *A. tumefaciens* strain B6 and the most resistant to natural crown gall infections. This result showed that sensitivity to cytokinin was a plant factor controlling tumorigenesis.

Additional keywords: hormone, host range, *iaa*, poplar, T-DNA mutants.

The crown gall disease is characterized by tissue overgrowths generally located on the crown and roots of dicots, gymnosperms, and some monocots (11). It is a major disease that is widespread in tree nurseries throughout the world, affects growth and commercial quality of stocks of many species (2), and causes important financial losses in orchard production (14,30). In some instances, breeders have abandoned species or cultivars that are too susceptible to crown gall (27). Such a decision is particularly serious when the plants are required for special purposes, such as aspen hybrids (*Populus tremula* × *P. alba*) that have been selected for forestation of hydromorphic soils (21). Crown gall is common in aspen nurseries (31), and various means of control have been attempted, including biocontrol with strain K84 and culling of diseased plants. However, biological control had no or very limited effects, probably because K84-resistant agrobacteria were already present in the nursery soil (X. Nesme, *unpublished data*). Culling was more efficient, but did not eradicate the disease (30). Thus, the selection of resistant aspens has also been considered.

Although resistant genotypes have been selected in several host species (3,26,27,36,47,54), plant traits that control tumorigenesis are poorly understood compared with present knowledge of virulence in *Agrobacterium* spp. (37,51). Most bacterial tumorigenic functions are encoded by a tumor-inducing plasmid or Ti plasmid (50). During the infection process, a fragment of this plasmid, called T-DNA, is transferred to the plant genome, where it is expressed like native plant genes (10). Transfer of T-DNA is controlled by virulence genes located in the nontransferred *vir* region of the Ti plasmid upon induction of compounds exuded by wounded plant cells (45). Oncogenes responsible for tumor growth reside in the T-DNA region and cause overproduction of auxin (*iaa*M and *iaa*H) and cytokinin (*ipt*) in transformed cells (1,17,42). Bacterial host range is mainly controlled by the Ti plasmid (19,23,48). Studies have shown that the *vir* genes or the T-DNA oncogenes, particularly the *ipt* gene, may modify the host range to some extent (5,16,52,53). Differences in resistance between hosts can, therefore, be caused by plant factors that interact with *vir* genes or T-DNA oncogenes (12,29,46).

In this paper, a large number of wild-type strains of *A. tumefaciens* were screened for their ability to define differences in susceptibility among closely related cultivars of *P. tremula* × *P. alba*. Research with mutant *A. tumefaciens* was undertaken to show whether *iaa* and *ipt* genes were required to define cultivar differences. Results indicated a role for *ipt*, that encodes the biosynthesis of a cytokinin in transformed plants. Thus, the sensitivity of host tissues to exogenous cytokinin was estimated by a leaf disk assay, and sensitivity to cytokinin was compared with crown gall susceptibility after artificial and natural infections.

MATERIALS AND METHODS

Bacteria. A large number of wild-type strains of *Agrobacterium* spp., provided by the Collection Française de Bactéries Phytopathogènes (CFBP; INRA, Angers, France), were used to empirically find which strains were useful in defining resistance differences among aspen cultivars. For maximum diversity, the tested strains included three species (*A. tumefaciens*, *A. rhizogenes*, or *A. vitis* [formerly named biovar 1, 2, 3, respectively; 4,32,40]) harboring Ti plasmids of different opine types. Strains were isolated from various hosts (Table 1). All the strains were tumorigenic after inoculations of five herbaceous plant species commonly used for this purpose. The tumorigenicity tests with the five herbaceous plant species showed that strains had different host ranges. Oncogenic mutants were also used to show whether

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iaa and *ipt* genes were required to define cultivar host range differences. These mutants had the chromosomal background of C58 (strain GV3101 [50]) and harbored derivatives of pTiB6S3, the Ti plasmid of the wild-type strain B6 (Table 1). They were a gift of J. P. Hernalsteens, State University, Brussels, and A. Reynaerts, Plant Genetic System, Gent, Belgium. Plate cultures were started from single colonies from freshly revived lyophilized cultures.

Plant materials. Aspen cultivars used in this study were closely related hybrids of *P. tremula* L. × *P. alba* L., obtained by Lemoine (21) (Table 2). The cultivars were chosen because they showed markedly different responses to natural infections (30). Also, 'Astria', a triploid hybrid that is intensively used for forestation in Germany, was included. The reliability of the resistance assessment was tested with aspens propagated either in vitro or in growth chambers under a 16 h of light/8 h of dark photoperiod, with light provided by fluorescent tubes at a photon flux density of 50 μE m^{-2} s^{-1}. Different temperature regimes (22 ± 1°C in vitro, 25 ± 1°C or 19 ± 1°C in growth chambers) were used, since this parameter can affect agrobacterial virulence (9). Shoot cultures were established on Murashige and Skoog (MS) medium (28) with half-concentrated mineral salts.

TABLE 1. Characteristics of *Agrobacterium* spp. strains used in virulence assays on aspen cultivars

Wild-type strain[a]					
CFBP no.	Other code	Host of origin	Species of *Agrobacterium*[b]	Opine type of the pTi[c]	Tumorigenicity[d]
Virulence group C58[e]					
1903	C58	*Prunus cerasus*	*tumefaciens*	Nopaline	5
296	111	Unknown	*tumefaciens*	Nopaline	5
449		*Prunus domestica*	*rhizogenes*	Agropine	5
1317		*Rubus idaeus*	*rhizogenes*	Nopaline	5
1804		*Prunus persicae*	*rhizogenes*	Nopaline	5
1840		*Populus alba*	*rhizogenes*	Nopaline	5
1873		*Dahlia* sp.	*tumefaciens*	Agropine	5
1932		*Prunus persicae*	*tumefaciens*	Nopaline	5
1933		*Malus pumila*	*tumefaciens*	Nopaline	5
1961		*Populus bolleana*	*rhizogenes*	Nopaline	5
1962		*Prunus mahaleb*	*rhizogenes*	Nopaline	5
2177		*Populus tremula* × *P. alba*	*tumefaciens*	Nopaline	5
2178	82-139	*Prunus avium*	*rhizogenes*	Nopaline	5
2326		*Prunus avium*	*rhizogenes*	Nopaline	5
2410	M22	*Populus* sp.	*tumefaciens*	Nopaline	5
2411	CG4	*Salix purpurea*	*tumefaciens*	Nopaline	5
2516		*Populus tremula* × *P. alba*	*tumefaciens*	Nopaline	5
	Bo542	*Dahlia* sp.	*tumefaciens*	Agropine	5
Virulence group B6					
2413	B6	*Malus* sp.	*tumefaciens*	Octopine	5
1935		*Rosa* sp.	*rhizogenes*	Nopaline	4
Virulence group 1904					
1904	AG20	*Vitis vinifera*	*tumefaciens*	Nopaline	2
1905	AG28	*Vitis vinifera*	*rhizogenes*	Nopaline	3
2179		*Vitis vinifera*	*vitis*	Nopaline	3
Virulence group 354					
354	B10	Unknown	*tumefaciens*	Nopaline	1
450		*Malus pumila*	*rhizogenes*	Unknown	3
452		*Malus pumila*	*tumefaciens*	Unknown	3
1930		*Malus pumila*	*rhizogenes*	Unknown	2
2412	A1B6	Unknown	*tumefaciens*	Octopine	3
Virulence group 1001					
1001	ATCC 13333	*Malus sylvestris*	*tumefaciens*	Octopine	4
2407		*Vitis vinifera*	*tumefaciens*	Octopine cucumopine	2

Mutant strain[f]	Relevant characters	Reference
Chromosomal background		
GV3101	Derivative of C58 cured of pTiC58	50
Plasmid		
pTiB6S3	Nonmutated control	35
pGV2250	Insertion mutant in the *ipt* gene	29
pGV2255	Deletion of *ipt*, 6a and 6b	20
pGV2215	*iaa*M and *iaa*H genes deleted	20
pGV2235	Only *ipt*, *ocs*, and Km^r in the T-DNA	20
pGV2282	Insertion mutant in the *iaa*M gene	17
pGV2274	Insertion mutant in the *iaa*H gene	17

[a] Strains were mainly collected by Collection Française de Bactéries Phytopathogènes (CFBP) collaborators (Ridé, Lopez, Prunier, Nesme, and Michel), otherwise the usual codes have been indicated.
[b] *Agrobacterium* species correspond to biovars.
[c] The pTi opine types were determined according to Petit et al. (34).
[d] Tumorigenicity indicates the number of plant species that formed tumors when the strain was inoculated to tomato, tobacco, *Datura stramonium* and *Kalanchoë tubiflora* seedlings, and carrot root disks.
[e] Virulence groups were determined in vitro with five selected aspen cultivars.
[f] *ipt* encodes cytokinin biosynthesis; *iaa*M and *iaa*H are required together for auxin biosynthesis; 6a is required for opine excretion; 6b is required for tumor morphology; and *ocs* is required for octopine biosynthesis. Km^r indicates resistance to kanamycin.

Inoculation procedures. Inoculations were performed in wounded, subapical, nonligneous internodes. For aspens generated in vitro, wounds were made with a needle and inoculated with a dense bacterial suspension optically adjusted to 3×10^8 cells ml^{-1}. Aspens cultivated in growth chambers or herbaceous plants grown in the greenhouse were pricked with a toothpick previously dipped in plate cultures. Carrot root disks were cultivated axenically on water agar, and inoculated with a dense bacterial suspension. Each strain-cultivar combination was tested with four plants inoculated at two to three sites, and each trial was replicated twice with the wild-type strains and three times with the mutants. Control inoculations were performed with sterile distilled water. Opines were detected in tumors according to the procedure of Petit et al. (34).

Percentage of crown gall. Percentages of crown galled plants in a naturally infected nursery were determined by visual inspection of aspen root systems once in 1984 and once in 1986. These two instances corresponded to cutting harvests of two successive campaigns of multiplication. The 1984/1986 multiplication campaign was performed with root material obtained from the previous 1982/1984 campaign, and used only healthy material selected after a drastic culling of the diseased material. Aspens were propagated in the nursery by the root cutting method (30).

Sensitivity to cytokinin. A leaf disk assay was used to estimate cultivar sensitivity to cytokinin. The fourth and fifth leaves beneath the apexes of aspens cultivated in growth chambers or in the greenhouse were surface-sterilized with 4 mM $HgCl_2$ for 20 min, rinsed twice in 17 mM $CaCl_2$ for 10 min, and then incubated for 1 h in sterile water. Disks, 8-mm in diameter, were punched between major veins and randomly plated on standard MS medium supplemented with carbenicillin at 500 mg $liter^{-1}$, Benlate (benomyl; du Pont, Boston) at 100 mg $liter^{-1}$, and BA (benzyladenine; Sigma Chemical Co., St. Louis) at 0.03 to 32 μM. BA solutions were obtained by twofold serial dilutions, and then they were filter-sterilized and added to MS medium after autoclaving. Sixteen to thirty disks per cultivar-BA concentration combination were used in two independent experiments. Sealed plates were incubated at 22 ± 1°C under a 16 h light/8 h dark photoperiod, with light provided by fluorescent tubes at a photon flux density of 50 μE m^{-2} s^{-1}. Leaf disks were scored for morphological changes twice a week for 3 months. The sensitivity to exogenous cytokinin was estimated by calculating the ED_{50} value, defined as the BA concentration causing necrosis on 50% of disks after 40 to 45 days.

Data analysis. Statistical analyses were performed according to Snedecor and Cochran (44) using the Stat View 4.0 software (Abacus Concepts, Inc., Berkeley, CA).

RESULTS

Resistance assessment of aspen cultivars using wild-type *Agrobacterium* spp. In initial experiments, strains of *Agrobacterium* spp. isolated from aspen crown galls (i.e., 1961, 2516, 2177, 1840, and 2410) formed tumors with every tested aspen cultivar. Therefore, a larger set of strains was screened to select those that could define differences in cultivar resistance in a reliable manner. Virulence screening of the 30 strains was performed with plants generated in vitro, because tumor formation is optimal in this condition. Since the manipulation of a large number of cultivars in vitro is difficult, five aspen cultivars were selected based on differences in susceptibility to natural infections. The 30 wild-type strains were divided into five groups of identical virulence, allowing for discrimination of four resistance groups among the five selected aspen cultivars (Table 3). The reliability of the resistance assessment of the five cultivars was tested in growth chambers and in vitro at various temperature regimes (19, 25, and 22°C, respectively) with virulence group representatives C58, B6, 1904, and 354. Reliable results were obtained with C58, B6, and 354, but not with 1904, which was very sensitive to temperature (data not shown). Therefore, the resistance of the remaining aspen cultivars was estimated in a growth chamber at 19°C with the three selected strains that gave reliable results. All aspen cultivars could then be ranked into three classes of resistance: susceptible, intermediate, and resistant (Table 4).

Relation with response to natural infections. The response to natural infections was estimated by the percentage of crown galled plants per cultivar determined in two instances (Table 5). Data were compared to the resistance rank of aspen defined by artificial infections, and a significant rank correlation was found (Kendall's rank correlation corrected for ties, $P = 0.016$). The Kendall's coefficient of correlation ($\tau = -0.301$) was rather small, however, suggesting that only a small part of the disease response was controlled by differential plant susceptibility to *A. tumefaciens*. Nevertheless, the most salient feature was that resistant aspens (did not form tumors with B6) consistently had the lowest percentage of crown galls (mean percentage = 4.5%). As a result, resistance to B6 could be considered as an indicator of the tolerance of aspen cultivars to crown gall under natural conditions.

Assays with T-DNA mutants of pTiB6S3. The following experiment was aimed at defining whether T-DNA oncogenes of B6 were involved in differences in susceptibility among aspen cultivars. For this purpose, aspen cultivars (selected for their differences in susceptibility to artificial infection) were inoculated with a strain harboring various derivatives of the Ti plasmid of B6 that were either *iaa*- or *ipt*-defective. Results showed that the aspen cultivars had different resistance patterns when inoculated with

TABLE 2. Pedigrees of the aspen (*Populus* spp.) cultivars used to find differences in susceptibility to crown gall

Cultivar[a]	Parent	
	P. tremula (female)	*P. alba* (male)
706-1	5820	5872
706-2	5820	5872
706-3	5820	5872
706-8	5820	5872
709-1	5813	5872
709-27	5813	5872
709-3	5813	5872
710-23	5814	5872
710-24	5814	5872
710-27	5814	5872
712-1	5815	5872
712-7	5815	5872
712-8	5815	5872
717-1-1[b]	5903	6072
717-1-2	5903	6072
717-1-B4	5903	6072
'Astria'	Unknown	*P. tremuloides*

[a] Cultivars were F_1 hybrids obtained from wild-type progenitors.
[b] The 717-1-1 hybrid is registered in the European list of poplar cultivars authorized for plantation under the name 'Rajane.'

TABLE 3. Resistance patterns of selected in vitro generated aspen plants to wild-type strains of *Agrobacterium* spp.

Cultivar	Virulence group[a]				
	C58	B6	1904	354	1001
717-1-B4	+[b]	+	+	+	–
712-7	+	+	+	+	–
710-23	+	+	+/–	–	–
709-3	+	+	–	–	–
710-24	+	–	–	–	–

[a] Tests were performed at 22°C with 30 wild-type strains in two independent experiments. Strains with identical virulence were grouped in virulence groups as described in Table 1.
[b] + indicates that 29 to 100% of inoculated sites formed tumors after 1 month; +/– indicates 8% formed tumors; and – indicates no tumors formed.

oncogene mutants (Table 6). Therefore, since mutant bacteria had the same bacterial background (i.e., strain GV3101), the bacterial ability to define resistance differences among aspen cultivars appeared to be mainly determined by the Ti plasmid, and especially T-DNA oncogenes.

Among the T-DNA oncogenes, the various *iaa*-defective mutants formed tumors with cultivar 710-23, but not with cultivars 709-3 or 710-24. This showed that *iaa* genes were not absolutely required to define resistance differences. On the other hand, mutants defective for cytokinin biosynthesis formed tumors and roots with all cultivars, including 710-24, which was resistant to the unmutated parental strain. Thus, a defective *ipt* gene did not abolish the tumorigenicity of strains harboring a pTiB6S3, but abolished the ability to define resistance differences between aspens. This suggested a role of the sensitivity to the *ipt* gene product, a cytokinin, in the individual plant ability to form tumors.

TABLE 4. Resistance assessment of aspen cultivars with selected strains of *Agrobacterium tumefaciens*

Cultivar	*A. tumefaciens* strain C58	B6	354
Susceptible			
717-1-B4	+[a]	+	+
712-7	+	+	+
706-2	+	+	+
706-3	+	+	+
'Astria'	+	+	+
Intermediate			
709-3	+	+	–
710-23	+	+	–
709-27	+	+	–
709-1	+	+	–
712-8	+	+	–
706-8	+	+	–
706-1	+	+	–
Resistant			
710-24	+	–	–
712-1	+	–	–
710-27	+	–	–
717-1-1	+	–	–
717-1-2	+	–	–

[a] Tests were performed in a growth chamber at 19°C in two independent experiments. + indicates the presence of tumors containing expected opines, and – indicates the absence of both tumors and opine at inoculation sites.

TABLE 5. Percentage of crown gall in aspen cultivars in a naturally infected nursery

Cultivar	Multiplication campaign 1982/1984	1984/1986
Susceptible[a]		
717-1-B4	42 / 160[b]	86 / 497
712-7	0 / 118	10 / 428
706-2	97 / 170	150 / 381
706-3	13 / 199	70 / 530
Mean percentage[c]	16%	
Intermediate		
709-3	102 / 108	9 / 18
710-23	4 / 75	13 / 198
709-27	19 / 58	7 / 181
709-1	27 / 173	16 / 142
712-8	17 / 191	3 / 363
706-8	45 / 171	44 / 241
706-1	132 / 189	31 / 239
Mean percentage	22%	
Resistant		
710-24	4 / 149	1 / 142
712-1	2 / 177	1 / 422
710-27	2 / 103	16 / 336
717-1-1	21 / 136	35 / 401
717-1-2	31 / 192	31 / 500
Mean percentage	4.5%	

[a] The classes susceptible, intermediate, and resistant were as defined in Table 4.
[b] Data indicates the number of crown galled plants out of the total number of plants assayed.
[c] Mean percentages calculated after arcsin $\sqrt{p}$ transformation.

Cytokinin sensitivity of aspen cultivars. An experiment was attempted to define intrinsic differences in sensitivity to exogenous cytokinin of aspen cultivars. For this purpose, aspen leaf disks were incubated on media containing various amounts of BA, a cytokinin. On BA-free medium, the leaf disks survived 60 to 70 days; they then became chlorotic and degenerated slowly without browning of tissues. On BA-supplemented media, disks turned brown after 30 days and died a week later. Striking differences in sensitivity were observed among aspen cultivars (Fig. 1), allowing for the determination of ED_{50} values, defined as the BA concentration that kills 50% of disks after 40 to 45 days (Table 7). Observations showed that aspens with low ED_{50} values often developed 1- to 4-mm calluses that, in most cases, later gave rise to roots on BA-free medium or on medium containing <1 μM BA (Mann-Whitney's test for callus production effect on ED_{50} corrected for ties, $P = 0.036$).

Relationship between sensitivity to cytokinin and susceptibility to crown gall. The susceptibility to *A. tumefaciens* strains could largely be explained by the sensitivity to cytokinin as estimated by ED_{50} values (Table 8). The only exception was cultivar 706-3 (Table 7). Callus production was also significantly related to the susceptibility to *A. tumefaciens* strains (Table 8), with the exception of cultivar 717-1-2 (Table 7).

The percentage of plants crown galled in a naturally contaminated nursery could also be explained by the ED_{50} values and by callus production (Table 8). In this case, the value of the Kendall's coefficient ($\tau = -0.31$) was rather small, suggesting that only a small part of the susceptibility to galling was controlled by the plant sensitivity to cytokinin.

DISCUSSION

In spite of its economic importance for many crops and extensive studies on genetic control, attempts to find intrinsic plant factors involved in the resistance to crown gall are rather rare. Cultivars resistant to *A. tumefaciens* have been described in several host plants including *Nicotiana* spp. (49), *Arabidopsis thaliana* (22), *Cucurbita maxima* (43), *Pisum sativum* (39), *Populus deltoides* (38), *Glycine max* (33), *Medicago sativa* (24), *Prunus* spp. (36), *Rubus idaeus* (54), *Chrysanthemum morifolium* (6,26), *Vitis* spp. (47), and *Rosa* spp. (3). In this list, only the latter six crops significantly suffer from crown gall outbreaks, and, even in these cases, there is no information about resistance of the se-

TABLE 6. Virulence of mutated *Agrobacterium tumefaciens* strains on aspen cultivars

Cultivar[a]	Plasmid content of strain GV3101[b]: *ipt*-deficient mutants (pGV2250, pGV2255)	Unmutated control (pTiB6S3)	*iaa*-deficient mutants (pGV2215, pGV2235, pGV2282, pGV2274)
710-23	+r	+	+b
709-3	+r	+	–
710-24	+r	–	–

[a] Assays were performed with plants generated in vitro at 22°C in three independent experiments.
[b] + indicates tumors; – indicates no tumors formed; b indicates the presence of buds associated with tumors formed by a strain harboring pGV2215; r indicates the presence of roots associated with tumors with the strains harboring pGV2250 and pGV2255.

lected material in natural conditions. Moreover, the study of plant factors involved in resistance often utilizes model host plants, which are generally not used agronomically. To our knowledge, no work has yet established a link between crown gall in experimental plots naturally contaminated by the pathogen with a resistance assessment done after artificial infections and the finding of an intrinsic plant factor correlated to crown gall susceptibility. Nevertheless, all these works suggest that resistant or tolerant cultivars can also be defined in other plant species for which crown gall is significantly important. This, in turn, supports the feasibility of using genetic control under conditions in which correlations can be established between artificial infection data and behavior under natural conditions, as long as the selection for resistance does not counterselect undesirable plant traits.

Agrobacterium spp. strains for use in reliable resistance tests were selected with the understanding that growth conditions and temperature affect agrobacterial virulence (9). Some bacterial strains were found to be highly sensitive to temperature for unknown reasons, and these bacteria were not used to define aspen resistance. On the other hand, there was no difference in susceptibility between plants generated in vitro and plants cultivated in growth chambers under any of the temperature regimes. Thus, the resistance of numerous aspen cultivars could be routinely defined in growth chambers with the three selected strains: C58, B6, and 354.

Among the selected strains, strain B6, which has been used to define resistance differences in several other species of host plants (6,26,39,43), has a particular agronomic interest, since aspen cultivars resistant to this strain consistently had the lowest rate of crown gall in naturally contaminated plots. Nevertheless, the field resistance obtained by a selection of cultivars resistant to B6 was not enough to get an acceptable control of the disease without sanitation practices. A drastic culling of the diseased material (30), or preferably the use of *Agrobacterium*-free plants generated in vitro (C. Ponsonnet and X. Nesme, *unpublished data*), must also be implemented to reach a crown gall percentage lower than 5 to 10%, a maximum acceptable level for a disease affecting plant quality (2). A higher level of resistance in aspen cultivars is, therefore, desirable. Resistance to C58 could serve as an indicator, since poplars of other sections which are generally not affected by crown gall are generally resistant to this strain (38).

Bacterial genes involved in defining resistance differences were studied using strain B6. It has been hypothesized that host-*Agrobacterium* incompatibility could be because of the inability of the bacteria to bind host cells (25), noninduction of virulence genes (41), or induction of a hypersensitive response caused by toxic overproduction of phytohormones at the inoculation sites by both *vir* and T-DNA genes (53). These various hypotheses were tested by inoculating mutant agrobacteria to aspens. As in several other systems (19,23,48), incompatibility to B6 appeared to be mainly determined by its Ti plasmid. Thus, it was unlikely that *Agrobacterium*-host cell binding was involved in aspen resistance, since this step is controlled by chromosomal genes (8,13). The incompatibility also does not result from a lack of induction or a toxic effect of *vir* genes, since one cultivar resistant to a strain harboring pTiB6S3 formed tumors with other strains carrying T-DNA mutants of the same plasmid having identical *vir* genes. On the other hand, T-DNA oncogenes, especially *ipt*, appeared to be strongly involved, since mutants retained their ability to define resistance differences as long as they had a functional *ipt* gene, and this property was abolished in the absence of this gene. Involvement of *ipt* in host range definitions has been described in the *A. vitis*/grapevine system. In this system, a functional gene from a virulent strain permitted expansion of the host range of the limited host range (LHR) strains which had nonfunctional *ipt* (6,16). It is not known, however, whether virulence differences among 354, B6, and C58 were because of differential expressions of their respective *ipt* genes.

Our results strongly implied that hormone sensitivity could control susceptibility to crown gall in aspens. Thus, a leaf disk

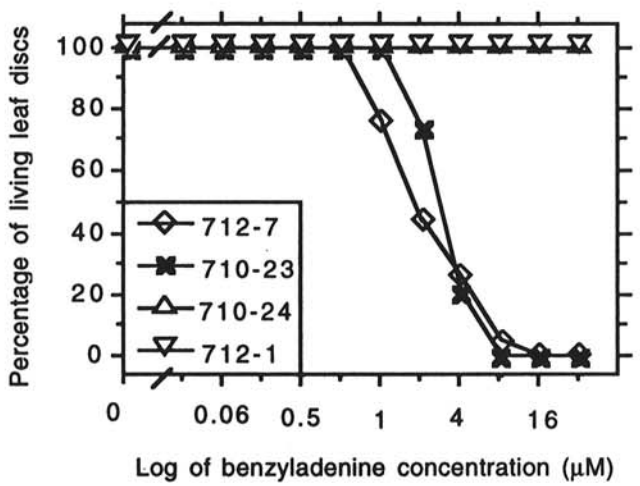

Fig. 1. Effect of the amount of exogenous benzyladenine (BA) on the survival of leaf disks in different aspen cultivars. Sensitivity assays were performed in vitro at 22°C with leaf disks plated on Murashige and Skoog medium containing various amounts of BA in two independent experiments. Data were scored after 40 to 45 days.

TABLE 7. Sensitivity to exogenous cytokinin of aspen cultivars

Cultivar	Sensitivity to cytokinin[a]	
	ED_{50}[b]	Callus production[c]
Susceptible[d]		
717-1-B4	2	+
712-7	2	+
706-3	16	+
'Astria'	2	+
Intermediate		
709-3	4	+
710-23	4	+
712-8	8	–
706-8	8	+
Resistant		
710-24	32	–
712-1	32	–
710-27	32	–
717-1-2	32	+

[a] Determined by a leaf disk assay in two independent experiments.
[b] The benzyladenine (BA) concentration killed 50% of leaf disks after 45 to 50 days.
[c] Callus production after 45 to 50 days; + indicates the presence of calli on leaf disks plated below 1 µM BA, generally developing into roots later; and – indicates no callus.
[d] Classes of resistance to *A. tumefaciens* strains are defined in Table 4.

TABLE 8. Correlation between sensitivity to cytokinin and susceptibility to crown gall of aspen cultivars[a]

Sensitivity to cytokinin	Susceptibility to selected strains of *A. tumefaciens* C58, B6, 354	Percentage of crown galled plants per clone in a naturally contaminated nursery
ED_{50}	$n = 12$ $\tau = 0.78$ ($\tau^2 = 60\%$) $P = 0.0004$	$n = 22$ $\tau = -0.31$ ($\tau^2 = 10\%$) $P = 0.043$
Callus production	$n = 14$ $P = 0.020$	$n = 26$ $P = 0.002$

[a] Effects of the variables ED_{50} value and callus production on aspen susceptibility to crown gall were tested by Kendall's rank of correlation and the Mann-Whitney *U* test, respectively, after eventual corrections for ties. Detailed data are available in Tables 4, 5, and 7.

assay was developed to measure cultivar sensitivity to exogenous cytokinin. Individual responses of closely related cultivars varied from 2 to 32 μM BA. We do not know, however, whether toxicity (which defines ED_{50} value) was a real property of cytokinins as it is for auxins (7), or if it was in a phenotype expressed only by surviving explants. ED_{50} values showed that resistant aspens were not very sensitive to cytokinin. Thus, our hypothesis was confirmed, even if exceptions to the rule indicated that other unknown plant factors must also control the phenomenon. Plant genotypes resistant to auxin are resistant to *A. tumefaciens* strains (15,22,49). However, to our knowledge, the involvement of the sensitivity to cytokinin in the control of susceptibility to crown gall has been hypothesized, but not clearly established. As suspected in other systems, resistant aspens seemed to require more cytokinin than susceptible cultivars to reach the cytokinin/auxin ratio required for tumor development (18).

This study showed that individual resistance to *A. tumefaciens* of aspen cultivars was largely related to hormonal sensitivity. This relationship probably occurs in many other host plants susceptible to crown gall. Studies of this relationship could facilitate the study of plant contribution to crown gall development, including, for instance, the host-mediated constraints to plant transformation with *A. tumefaciens*.

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