# Effect of Host Plant Genotype and Growth Rate on *Agrobacterium tumefaciens*-Mediated Gall Formation in *Pinus radiata*

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## ABSTRACT

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Effect of host genotype on *Agrobacterium tumefaciens*-mediated gall formation in *Pinus radiata* was examined using clonal tissue culture shoots and rooted plantlets. Tissue culture shoots were inoculated in vitro with *A. tumefaciens* strain 542 or C2/74, both known to be highly infective in pines. Rooted plantlets of the identical genotypes used in vitro and seedlings from the same seedlot were established in the greenhouse and inoculated with strain 542. Gall formation frequency of tissue culture shoots and rooted plantlets was genotype dependent. Rooted plantlets and seedlings of the same age did not differ in susceptibility to *A. tumefaciens* strain 542. In vitro gall formation frequency did not predict greenhouse gall formation frequency. Higher gall formation frequencies were found in rooted plantlets in the greenhouse as compared to tissue culture shoots of the same clones. Gall formation occurred earlier and over a shorter period with in vitro as compared to greenhouse inoculation. Galls on seedlings and tissue culture shoots were spherical and succulent, whereas rooted plantlets had less localized and woodier galls. The correlation between mean growth rate at the time of inoculation and gall formation frequency was significant for rooted plantlets. When only the more rapidly growing rooted plantlets within each clone (top 50%) were considered, gall formation frequency was higher than for the more slowly growing individuals within the same clones, and the effect of genotype was obscured. Overall, 58% of gall tissue had detectable opine levels. An inverse relationship existed between the woodiness of gall tissue and the amount of opine detected. Our data are consistent with the idea that a major component of the host-pathogen interaction is the genetic and environmental control of host plant cell division at the time of inoculation.

The largest fraction of the world's wood supply is provided by *Pinus* species, and *Pinus radiata* is one of the most important species. Genetic improvement of pine species using classical breeding methods is slow because of the long generation and testing times. Genetic engineering could provide an alternative route to genetic improvement if methods for gene transfer could be developed (34).

*Agrobacterium* has been used extensively to transfer genes into many dicotyledonous plants (4,10) and a few monocots (7,30,33). Susceptibility to *Agrobacterium* has long been known in gymnosperms (11,36) and has been shown in the most economically important group of conifers, the pines (35). Recent work with *Larix decidua* has shown that virulent *A. rhizogenes* can be used to produce transformed plants (19). Clearly, there is potential for using *Agrobacterium* species to produce transgenic pine plants.

Development of an *Agrobacterium*-mediated gene transfer system is facilitated by screening host plant and bacterial genotypes to identify the combination giving the highest levels of infectivity (5,13,39). Previous investigations have demonstrated the importance of such interactions with dicots (22), *Brassica* species (15), legumes (17), and conifers (8,12,25,36,38). Host genotype at the cultivar level also has been shown to be significant in grape (21), soybean (6,27,28), and pea (14).

It is not possible to screen for genotypes of forest tree species for high rates of *Agrobacterium* infectivity by using cultivars or inbred lines because such genetic populations do not exist. Other levels of genotypic definition, open- and control-pollinated families, are available for infectivity screening in economically important species for which breeding programs have been established. Several studies have shown significant effects of half- and full-sib families on *Agrobacterium* infectivity. The significance of half-sib family influence on susceptibility to *Agrobacterium* infection has been demonstrated in *Picea abies* (9). The use of control-pollinated hybrid poplar families showed that family, and the female parent in particular, significantly influenced susceptibility to *Agrobacterium* (32). Studies of *Agrobacterium*-induced gall formation on fixed genotypes through the use of clonal plants are rare. Clonal material was used in a study of *Agrobacterium* infectivity with the forest tree species *Alnus glutinosa, A. incana,* and *Betula papyrifera*; however, the study was not designed to address clonal genotype influence per se (24). The present study was specifically designed to examine the influence of fixed genotypes (clones) on the susceptibility of *P. radiata* to *A. tumefaciens*.

Three other factors known to influence gall formation frequency following *Agrobacterium* stem inoculation also were examined. Hobbs et al (14) reported that no correlation was found between transformation frequency in vivo and in vitro when the same pea genotypes were inoculated. Both in vitro (23) and in vivo (35,38) stem inoculations have been used successfully to produce galls in pines. We used both in vitro and in vivo stem inoculations to test the rates of gall formation using the same seven clones in each system. Seedling age influences gall formation according to work with *Picea* species (12,16) and *Pinus* species (38). To test age effects, gall formation frequencies were compared between seedlings grown in the greenhouse and clonal greenhouse plants derived from tissue culture, which are thought to be ontogenetically older (18). Finally, it has been pointed out that cell cycle phase may be a common denominator in influencing gene transfer efficiency in several systems (37). We examined genotype growth rate at the time of inoculation to determine if growth rate is a significant factor influencing frequency of gall formation.

## MATERIALS AND METHODS

**Plant material.** Three populations of *Pinus radiata* D. Don plant material were used in the experiments: unrooted, adventitious tissue culture shoots, rooted plantlets, and seedlings. The seed source used for all plant material was bulk seedlot 102 from the *P. radiata* breeding program of the Forest Research Institute, Rotorua, New Zealand. The procedures of Aitken-Christie et al (1) were used to produce and root the adventitious shoots. Briefly, the seeds were pretreated following Reilly and Washer (31). Embryos were aseptically excised and inverted into meristem initiation medium (LP5 in [1]) with the cotyledons half submerged. Cotyledons were removed from the embryo 3 days later and returned to the same medium where they remained for 3 wk to

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produce meristematic tissue. Cotyledons with proliferating meristematic tissue were transferred to shoot elongation medium (LP0 in [1]), and subcultured to fresh medium every 4 wk. As shoots organized and elongated, they were excised and grown separately. When shoots were 4 cm in height, they were either used to produce rooted plantlets for the greenhouse experiment or were transferred to fresh LP0 medium, four or five to a 10-cm Magenta box (Magenta Corp., Chicago), for 1 wk in preparation for in vitro inoculation. Eighteen of the original 100 embryos (clones) produced enough shoots after 12 wk in culture for experimental use.

Root meristems were induced by growing shoots on 1.0% (w/v) water agar containing 1.0 mg/L of indolebutyric acid and 0.5 mg/L of naphthaleneacetic acid for 10 days. After root induction, shoots were transferred to flats in the greenhouse and kept under intermittent mist for 8 wk. The medium was 1:2:2:1 peat/perlite/vermiculite/sand. All tissue cultures were grown at 23 C under a 16-h photoperiod supplied by Gro-lux wide spectrum fluorescent lights at 40 $\mu molm^{-2}s^{-1}$.

Seedlings were produced from seeds sown at the time in vitro shoots were first transferred to the greenhouse to establish roots. Seedlings and plantlets were transplanted to standard Leach tubes (Cone-tainer Nursery, Canby, OR) after 12 wk of greenhouse growth. After an additional 12 wk, seedlings and plantlets were transplanted from the Leach tubes to 10- × 10- × 35-cm-deep containers ("treepots," TreePot Enterprises, Garden Grove, CA) 4 wk before inoculation. Medium for rooting and all transplants was 1:2:2:1 peat/perlite/vermiculite/sand. Incandescent lights providing 50 $\mu E\ m^{-2} s^{-1}$ and a 24-h photoperiod were used between 1 October and 30 April to promote continued growth. Rooted plantlets and seedlings were watered and fertilized (Peter's 20-19-18) as needed to maintain rapid growth.

**Inoculation.** The *A. tumefaciens* strains were obtained from Larry Moore, Oregon State University, and maintained in 30% glycerol stocks at −70 C. Cultures were grown on potato-dextrose agar (3.7%, w/v) for 3 days before inoculation. In vitro shoots were inoculated with the virulent strains 542 and C2/74 and avirulent strain A136, included to test the effect of bacteria on wound tissue growth; greenhouse-maintained rooted plantlets and seedlings were inoculated with strains 542 and A136 only. These strains were used because in previous studies (A.-M. Stomp, *unpublished data*) high susceptibility was observed using strain 542 in *P. radiata* (38) and strain C2/74 in *P. taeda*. In vitro and greenhouse inoculations were accomplished by dipping the tip of a no. 11 scalpel blade into the *A. tumefaciens* culture and stabbing the stem of each shoot or plant seven times as close to the apex as possible and deeply enough to penetrate the xylem tissue. Stabbing the stem with a sterile blade served as the "inoculation" for the wound-only controls.

Uniform tissue culture shoots were inoculated when approximately 4.5 cm tall. After inoculation, all shoots were transferred every 2 wk to fresh medium containing 500 mg/L of carbenicillin to kill free-living *A. tumefaciens*.

**Experiment design and data analysis.** Two factorial experiments were done, one in tissue culture and the other in the greenhouse. In the tissue culture experiment the treatments were genotype at seven levels (clones) and *Agrobacterium* strain at four levels (including wounded only controls). The seven clones were selected based on the fact that each had a sufficient number of healthy, uniform shoots at the time inoculations were done. The two virulent *A. bacterium* strains 542 and C2/74 were each used to inoculate 10–12 shoots per clone (two boxes of five shoots each or three boxes of four shoots each). The avirulent strain A136 was used to inoculate eight shoots per clone (two boxes of four shoots each), and five shoots per clone (one box of five shoots) were wounded only controls. Boxes containing the treated shoots were placed randomly on the growth chamber shelf. The experiment was repeated 8 wk after the original experiment was initiated. Thus, a total of 488 tissue culture shoots were used: an average of 44 shoots per clone inoculated with virulent strains (306 total), 16 shoots per clone inoculated with the avirulent strain (112 total), and 10 shoots per clone (70 total) used as wounded-only controls.

In the factorial greenhouse experiment, the treatments were genotype in eight levels (clones), including six clones that were used for in vitro inoculation, and *Agrobacterium* strain in three levels (including wounded only controls). An additional genotype level consisting of a population of seedlings of mixed genotype, but of the same seedlot used to produce the rooted plantlets, also was included. The number of rooted plantlets depended on the tissue culture and rooting success of each clone. Eight clones in the greenhouse experiment were represented by enough individuals for direct clonal comparisons, but a few individuals were included from an additional 10 clones so that a wider genetic base would be represented when gall formation frequency in rooted plantlets and seedlings was compared. The three levels of *A. tumefaciens* strain treatment were the virulent 542 strain, the avirulent A136 strain, and wounded-only controls. Strain 542 was used to inoculate between 10 and 12 plantlets from each of the eight more numerous clones, two or three plantlets from each of the 10 additional clones, and 31 seedlings. The avirulent *A. tumefaciens* strain A136 was used to inoculate a genotype mix of 15 rooted plantlets and 15 seedlings. A genotype mix of 15 rooted plantlets and 15 seedlings was wounded-only controls. The experiment was repeated 8 wk after the original experiment was inititiated. The only difference between the original and repeat experiment was that 64, rather than 31, seedlings were inoculated with strain 542 in the second. Thus, a total of 261 rooted plantlets and 155 seedlings were used. Of the rooted plantlets, 201 were inoculated with the virulent strain, 30 were inoculated with the avirulent strain, and 30 were wounded only. Of the seedlings, 95 were inoculated with the virulent strain, 30 were inoculated with the avirulent strain, and 30 were wounded only.

Tissue culture shoots, rooted plantlets, and seedlings were evaluated for the presence of galls at different times after inoculation. Tissue culture shoots were examined every 2 wk starting 2 wk postinoculation and continuing through week 18. Rooted plantlets and seedlings were examined frequently until the first galls appeared at week 6. Subsequent gall assessments were made 20 and 32 wk after inoculation. These data were used to calculate gall formation frequency. Rooted plantlet and seedling heights were measured every 2 wk starting 4 wk before inoculation and ending 6 wk after inoculation. Plant height data were used to calculate growth rate at the time of inoculation. This value included the time from 2 wk before to 2 wk after inoculation. Only the eight more numerous clones in the greenhouse experiment were used to analyze the effect of genotype on gall formation frequency and growth rate at the time of inoculation because they were represented by enough individuals to provide a sufficiently robust statistical test. All data were statistically analyzed using SAS (PROC FREQ, PROC GLM, Duncan's LSD, and PROC CORR, SAS Institute, Cary, NC). In no case were results found to be different between the original and the repeat of either the tissue culture or the greenhouse experiments. Thus, the values presented in the results are pooled within each experiment across the original and repeat.

**Opine analysis.** Presence of agropine and mannopine was assayed using rooted plantlets and seedlings to verify gene transfer in putative gall tissues. Galls, nongalled stem segments at the point of wounding (strain 542, strain A136, and wounded only controls), and stem segments away from the point of wounding (galled and nongalled individuals) were excised from rooted plantlets and seedlings at 24 and 32 wk postinoculation and frozen. Gall tissue was extracted and electrophoresed following the procedures of Sederoff et al (35). Samples that resulted in faint spots were rerun using 10× concentration of the original extract if enough remained after the original run.

## RESULTS

Most galls in the tissue culture experiment formed within 4–6 wk after inoculation, with only clone 17 developing galls as late as week 12. Galls continued to grow through week 18 and attained sizes ranging from 0.2 to 1.0 cm in diameter (Fig. 1A). The first galls were observed on rooted plantlets and seedlings at 6 wk, but most were not apparent until 10 wk or more after inoculation.

New galls were first observed in the greenhouse as late as 32 wk after inoculation. Because galls were sloughed off in some cases, the frequency of greenhouse gall formation decreased between week 20 (39%) and week 32 (32%). None of the tissue culture shoots, rooted plantlets, or seedlings in the greenhouse treated as wounded only controls showed any swelling or gall formation. When each experiment was repeated, no difference was observed in the date of onset of gall formation or in frequency of gall formation.

Mean frequency of gall formation in the greenhouse at 20 or 32 wk for the 18-clone population of rooted plantlets did not differ significantly from that of seedlings. Gall frequencies 20 wk postinoculation were 38 and 39% for the rooted plantlets and seedlings, respectively, and 29 and 33%, respectively, after 32 wk. Galls on seedlings were green, succulent, distinct spheres up to 0.6 cm in diameter (Fig. 1B), whereas galls on clonal trees were brown, woody, swollen areas measuring up to 2.5 cm in diameter (Fig. 1C).

The avirulent *A. tumefaciens* strain A136 never induced gall formation and was therefore significantly different from the gall-inducing virulent strains 542 and A136 in the tissue culture experiment and strain 542 in the greenhouse experiment. The two virulent strains were not significantly different in gall formation frequency in tissue culture shoots. Strains 542 and C2/74 proved equally capable of inducing gall formation ($P = 0.61$). This is not surprising because the strains were chosen specifically for their high infectivity of pines. In the tissue culture experiment the absence of significant clone by virulent strain interaction ($P = 0.93$) indicated that clones performed consistently within the two strains.

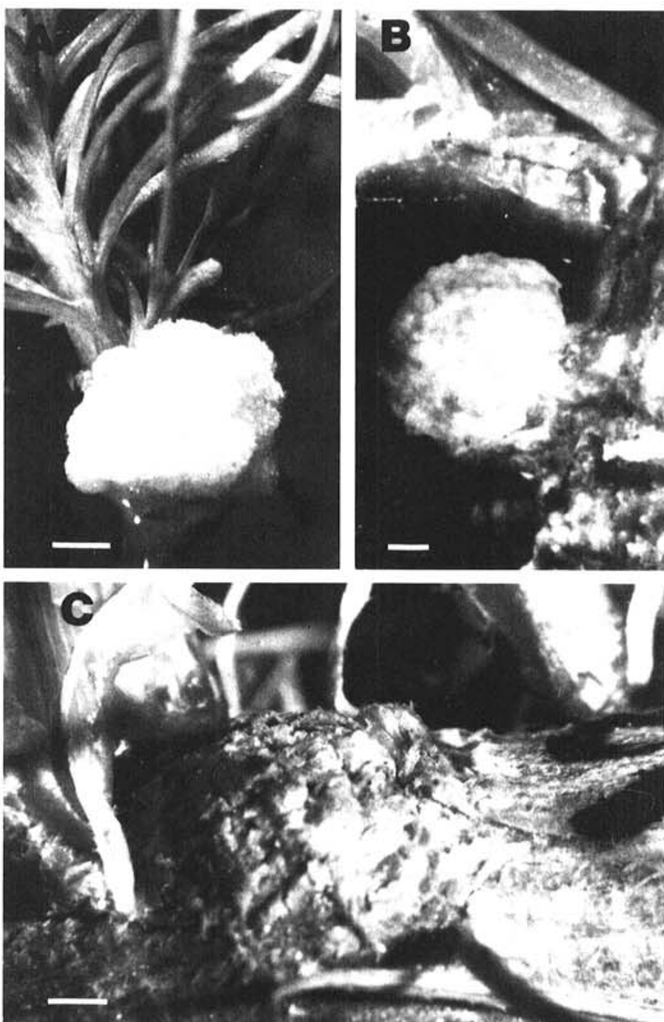


**Fig. 1.** Stem galls on *Pinus radiata* induced by inoculation with *Agrobacterium tumefaciens* strain 542, **A,** in vitro at 14 wk (bar = 2.5 mm); **B,** in the greenhouse on a seedling at 20 wk (bar = 1.0 mm); and **C,** in the greenhouse on a rooted plantlet at 20 wk (bar = 2.5 mm).

Across the six clones used in both the tissue culture and greenhouse experiments, gall formation frequency was lower for tissue culture shoots (14%) than for rooted plantlets (37% after 20 wk). Five of the six clones inoculated under both conditions had significantly greater gall formation frequencies for rooted plantlets as compared to tissue culture shoots (Table 1). Genotype was a significant factor in frequency of gall formation in tissue culture shoots and rooted plantlets (Table 1). Frequency of gall formation using tissue culture shoots did not accurately predict gall formation frequency when rooted plantlets were used. Clonal ranks varied considerably between the tissue culture experiment and the greenhouse experiment. An exception was clone 17, which had high gall formation frequencies for tissue culture shoots (39% across strains) and rooted plantlets (38 and 63% after 20 and 32 wk, respectively).

Positive among- and within-genotype associations were found between growth rate at the time of inoculation and gall formation frequency in rooted plantlets and seedlings. Eight clones and seedlings were considered in the analyses used to examine the relationship between growth rate at the time of inoculation and gall formation frequency because these were the only ones with enough individuals to provide reliable tests. Clones that were growing more rapidly at the time of inoculation tended to have higher gall formation frequencies (Fig. 2A). When seedlings and rooted plantlets within each clone were divided into two groups, the 50% faster-growers versus the 50% slower-growers at the time of inoculation, it was found that the fast-growers had significantly more galls for seven of eight clones. All the galls in clones 5 and 7 were formed on the faster-growing trees (Fig. 2B and C), and only clone 1 and seedlings did not differ significantly in gall formation frequency between fast-growers and slow-growers. Genotype significantly affected the frequency of gall formation 20 and 32 wk (data not shown) after inoculation in the more slowly growing individuals within each clone but not in the more rapidly growing individuals (Fig. 2B and C). Significant positive correlations (Pearson) between clone and seedling means for growth rate at the time of inoculation and gall formation frequency were found when considering all plants or the 50% slower-growers within each clone and seedlings but not when considering only the 50% faster-growers within each clone and seedlings. Similar but not as strong correlations were found for week 32.

TABLE 1. Gall formation frequency in *Pinus radiata* clones inoculated as tissue culture shoots and rooted plantlets with strains of *Agrobacterium tumefaciens*

| | Gall formation frequency (%)[w] | | | |
|---|---|---|---|---|
| | Strain C2/74 | Strain 542 | | |
| Clone | Tissue culture shoots | Tissue culture shoots | Rooted plantlets | |
| 1 | 7 ab[x] | 20 a–c | 41 a | **[y] |
| 4 | 38 a | 33 ab | NT[z] | |
| 5 | NT | NT | 14 c | |
| 6 | 13 ab | 13 bc | 26 a–c | * |
| 7 | NT | NT | 25 bc | |
| 8 | 16 ab | 11 bc | 29 a–c | * |
| 11 | 0 b | 0 c | 36 ab | ** |
| 14 | 0 b | 10 bc | 40 ab | ** |
| 17 | 33 a | 44 a | 38 ab | NS |

[w] Gall formation frequencies were calculated for tissue culture shoots inoculated with strains 542 or C2/74 after 18 wk and rooted plantlets inoculated with strain 542 after 20 wk. Values shown are combined data from the two repetitions of the tissue culture and greenhouse experiments.

[x] Values within a column followed by the same letter are not different at the $P = 0.05$ according to Duncan's LSD test.

[y] ** and * indicate differences between gall formation frequencies in tissue culture shoot and rooted plantlets inoculated with strain 542, using pairwise comparisons within clone, at the $P = 0.01$ and $P = 0.05$ significance levels, respectively. NS = not significantly different.

[z] NT indicates that the clone was not tested.

Thirty-four of the 58 galls (58%) had detectable levels of opines. Opines were detected in only 50% of gall tissue of the 32-wk-old seedlings and 47% of similar tissue of the 32-wk-old rooted plantlets (Table 2). However, 100% of positive gall tissue of the 24-wk-old seedlings had detectable opine levels (Table 2). Runs with the 10× concentrated extracts from the galls on the rooted plantlets showed that they had lower detectable opine levels than those on seedlings of the same age (Table 2). Within the 34 galls with detectable opines, 22 had agropine and mannopine, 11 had agropine only, and one had mannopine only, the expected opines using strain 542. No particular trend for opine type was observed by tree type, i.e., rooted plantlet or seedling.

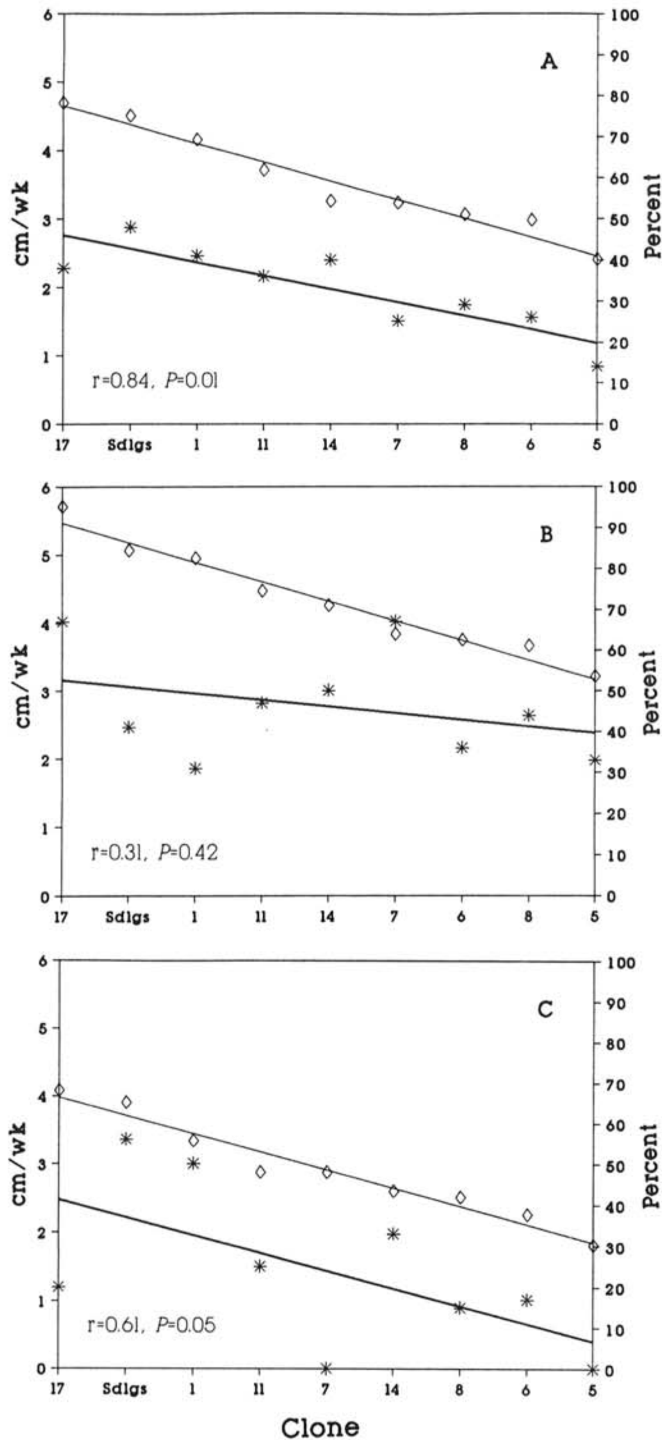


**Fig. 2.** Growth rate (cm/wk -◇-) of *Pinus radiata* rooted plantlets and seedlings at the time of inoculation with *Agrobacterium tumefaciens* strain 542 and percent gall formation (-*-) using **A,** all rooted plantlets and seedlings; **B,** faster-growing rooted plantlets and seedlings; **C,** slower-growing rooted plantlets and seedlings. Faster and slower refer to the top 50% and bottom 50%, respectively, of the individuals within each clone and seedlings with regard to growth rate at the time of inoculation. Clones are arranged in decreasing order according to growth rate at the time of inoculation. Each figure shows the Pearson correlation coefficient, *r,* between clonal means for growth rate at the time of inoculation and gall formation frequency and the probability, *P,* that it is significant. The values shown are combined data from the two replications of the greenhouse experiment.

The presence of detectable levels of opines in gall tissue appeared to be related to gall age and morphology. Seedling galls were green and succulent and were all found to have high levels of opine expression 24 wk after inoculation in the greenhouse. However, seedling galls were more woody and had detectable levels of opines in only 50% of the galls when assayed 32 wk after inoculation. Galls from the rooted plantlets, which were drier and woodier than galls which developed on seedlings, also exhibited opines in slightly less than half of the galls assayed. Of the 18 galls that expressed opines in the rooted plantlets, 10 did so at very low levels.

## DISCUSSION

Many studies have shown host genotype to be an important factor in frequency of gall formation following inoculation with *Agrobacterium*. We observed significant differences in gall formation frequency among genotypes (clones) when using either tissue culture shoots or rooted plantlets. Higher taxonomic levels have been used with other forest tree species to show that susceptibility to *Agrobacterium* (gall formation) was significantly influenced by species (8,36,38) and by family (9,32). Studies with crop plants show that cultivars can vary significantly in susceptibility to *Agrobacterium* (6,14,22,27,28).

Our results show that growth rate at the time of inoculation is an important factor in determining gall formation in *P. radiata*. Similar, informal observations have been made previously with conifers. Stomp et al (38) found that the highest gall formation frequencies in pine seedlings were obtained by inoculating young, elongating epicotyls just big enough to grasp for inoculation and capable of surviving initial wounding. Seedlings used in that study had rapidly growing, green succulent epicotyls, consistent with the idea that growth rate at the time of inoculation is a factor in *Agrobacterium* susceptibility in pines. Similarly, Ellis et al (12) observed reduced gall formation frequency in woody tissue of spruce seedlings as compared to succulent tissue, and speculated that this "age effect" could be the result of active growth in succulent tissue.

The genotypic influences we expected to find on gall formation frequency could be at least partially explained by growth rate at the time of inoculation. A strong, positive correlation was observed between average growth rate at the time of inoculation and gall formation frequency for rooted plantlets and seedlings. We conclude that growth rate at the time of inoculation can obscure genotype influence because gall formation frequencies for the fastest-growing individuals (top 50% within each clone) do not vary significantly among clones, and the correlation between growth rate at the time of inoculation and gall formation frequency is not significant. Above a certain growth rate at the

TABLE 2. Opine detection in galls produced on *Pinus radiata* stems by *Agrobacterium tumefaciens*[x]

| | Number tested | High level | | Low level[y] | | None | |
|---|---|---|---|---|---|---|---|
| | | (no.) | (%) | (no.) | (%) | (no.) | (%) |
| Rooted plantlets[z] | 38 | 8 | 21 | 10 | 26 | 20 | 53 |
| Seedlings (32 wk) | 8 | 4 | 50 | 0 | 0 | 4 | 50 |
| Seedlings (24 wk) | 12 | 12 | 100 | 0 | 0 | 0 | 0 |
| Total | 58 | 24 | 41 | 10 | 17 | 24 | 41 |

[x] Presence of opines was determined for galls formed on rooted plantlets and seedlings in the greenhouse after inoculation with strain 542.

[y] Opine level was considered low if it was detectable only by using a 10× concentration of the gall extract.

[z] Galls from rooted plantlets were all sampled 32 wk after inoculation. galls from seedlings were sampled 32 or 24 wk after inoculation.

time of inoculation, gall formation can occur at a frequency independent of genotype. Examining the more slowly growing individuals reveals significant differences among clones in gall formation frequency and a positive correlation between growth rate at the time of inoculation and gall formation frequency.

Our finding that growth rate at the time of inoculation and genotype is associated major determinants in gall formation frequency may explain some of the differences seen in *Agrobacterium* susceptibility in inbred crop plant cultivars. It has been demonstrated that genotype is an important determinant in host-pathogen interaction (3). We would predict that cultivar differences in growth rate at the time of inoculation would correlate with gall formation frequencies. We cannot test this prediction because crop plant screening studies do not present relative growth rates for the cultivars at the time of inoculation.

Finding a lack of consistency when looking at clonal means or rankings of tissue culture shoots as compared to rooted plantlets suggests that the inoculation system is also an important factor determining gall formation frequency. Differences among clones in growth rate at the time of inoculation could account for the differences in clonal means and rankings both within and between tissue culture and rooted plantlet populations. Tissue culture shoots were growing much more slowly than rooted plantlets at the time of inoculation, which could account for the differences in gall formation frequencies between tissue culture shoots and rooted plantlets of the same clone. Our highest gall formation frequencies were lower than the 86% reported with *A. tumefaciens* strain 542 for *P. radiata* by Stomp et al (38). The seed source, inoculation conditions, and age of inoculated plants were different in the system reported here, which could account for the discrepancy. The rooted plantlets and seedlings in this work were considerably older, a factor known to have a strong influence on gall formation. The differences in clone ranking between tissue culture shoots and rooted plantlets could result from different genotype responses to tissue culture versus greenhouse conditions. We do not have growth rate data at the time of inoculation for tissue culture shoots, so we cannot test this prediction with our data set. There could also be a difference in endogenous plant growth regulator content between rootless shoots in vitro and intact trees in the greenhouse, which could be another factor influencing frequency of gall formation.

In a recent review article, Potrykus (29) argued that "an appropriate wound response" is necessary for transformation. We have used an intact plant system to obtain results that suggest that rapid growth increases the frequency of transformation in *P. radiata*. These two ideas may be symptomatic of an underlying requirement for cell division in transformation. Consistent with this idea, An (2) observed that the highest number of stable transformation events were obtained during the logarithmic growth phase of tobacco callus, when the highest proportion of callus cells would be cycling through division. Similar findings were obtained with loblolly pine callus (A.-M. Stomp, *unpublished data*). Studies using electroporation of synchronized protoplasts (26) and microprojectile bombardment of synchronized cells (20) have shown that cells in M-phase have the highest rates of transient expression and stable transformation. Kartzke et al (21) also showed that the cell cycle stage influences DNA integration events. Interestingly, Mackay et al (24) found that a "preconditioning" of *Alnus* and *Betula* shoots on auxin-containing medium enhanced tumor formation in an in vitro inoculation system. Auxin is used to promote callus proliferation in these species. These studies and our data are consistent with the hypothesis that any genotype by environment interaction that increases cell cycling, including greenhouse practices promoting rapid growth, preconditioning of the plant tissue with auxin, or the genetically controlled rapid onset of wound callus formation, will increase the frequency of stable integration of T-DNA.

Production of transgenic pine plants remains elusive. We found that a rapid growth rate at the time of infection with *A. tumefaciens* is important to obtain high rates of successful gene transfer to pine cells. This knowledge will be useful in future attempts to achieve *Agrobacterium*-mediated transformation in pine.

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