Tolerance to the Herbicide Glufosinate in Transgenic Cranberry (*Vaccinium macrocarpon* Ait.) and Enhancement of Tolerance in Progeny

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ABSTRACT. The American cranberry (*Vaccinium macrocarpon* Ait.) was genetically transformed with the *bar* gene, conferring tolerance to the phosphinothricin-based herbicide glufosinate. Plants of one 'Pilgrim' transclone grown under greenhouse conditions were significantly injured by foliar treatments of 100 mg·L^{-1} glufosinate, although the injury was less severe when compared to untransformed plants. However, the same transclone grown outdoors in coldframes survived foliar sprays of 500 mg·L^{-1} glufosinate and higher, while untransformed plants were killed at 300 mg·L^{-1}. Actively growing shoot tips were the most sensitive part of the plants and at higher dosages of glufosinate, shoot-tip injury was evident on the transclone. Injured transgenic plants quickly regrew new shoots. Shoots of goldenrod (*Solidago* sp.) and creeping sedge (*Carex chordorrhizia*), two weeds common to cranberry production areas, were seriously injured or killed at 400 mg·L^{-1} glufosinate when grown in either the greenhouse or coldframe environment. Stable transmission and expression of herbicide tolerance was observed in both inbred and outcrossed progeny of the above cranberry transclone. Expected segregation ratios were observed in the outcrossed progeny and some outcrossed individuals demonstrated significantly enhanced tolerance over the original transclone, with no tip death at levels up to 8000 mg·L^{-1}. Southern analysis of the original transclone and two progeny selections with enhanced tolerance showed an identical banding pattern, indicating that the difference in tolerance levels was not due to rearrangement of the transgene. The enhanced tolerance of these first generation progeny was retained when second generation selfed progeny were tested.

The American cranberry is a low-growing perennial vine, grown commercially in solid stands in beds up to 2 ha in size. Newly planted vines require 3 to 5 years to establish a good cover and to begin producing average yields; however, they may be productive for many decades thereafter. Weed competition can significantly reduce cranberry yields (Dana, 1989; Patten and Wang, 1994), and growers must constantly utilize cultural and chemical measures to control the weeds. Because of the high requirement for water during various phases of production, most cranberry beds are located near major water resources, thus increasing the potential for negative environmental impacts from weed control practices. The use of biorational herbicides that have broad spectrum activity, low toxicity to wildlife, and low half-lives in the soil, combined with cranberry plants genetically modified for tolerance to these herbicides, offers an attractive weed control strategy.

L-Phosphinothricin is a naturally occurring amino acid that is a potent inhibitor of all forms of glutamine synthetase occurring in plants (Leason et al., 1982; Wild and Manderscheid, 1984). Inhibition of glutamine synthetase results in the toxic accumulation of ammonia produced by direct uptake, photorespiration, nitrate reduction, or protein/amino acid metabolism (Wallsgrove et al., 1987; Wild et al., 1987). Tolerance to the herbicide has been obtained in many crops and often employs the resistance gene, *bar*. *Bar* was derived from a common soil bacterium *Streptomyces hygroscopicus* and encodes an enzyme that acetylates phosphinothricin, thus inactivating the herbicidal activity (Thompson et al., 1987).

This paper reports the successful transformation of cranberry with the *bar* gene. One transclone showed moderate tolerance to the glufosinate (the ammonium salt of phosphinothricin) herbicide Liberty, and some outcrossed progeny showed significantly elevated tolerance over the original transclone, reaching commercially useful levels.

Materials and Methods

GENETIC TRANSFORMATION OF CRANBERRY WITH THE *bar* GENE. The transformation protocol was identical to that previously described for the transfer of *gus* and *bt* genes into cranberry (Serres et al., 1992). The cultivars 'Stevens' and 'Pilgrim' were used. The construct had a pUC19 plasmid backbone (Yanish-Perron et al., 1985) and contained the *bar* gene driven by a cauliflower mosaic virus (CaMV) 35s promoter (Nagy et al., 1985; Odell et al., 1985) containing the alfalfa mosaic virus noncoding leader sequence (Gehrke et al., 1983) 5' to the coding sequence and a soybean small subunit gene polyadenylation signal region (Berry-Lowe et al., 1982). The plasmid also contained the *aphII* gene, driven by the nopaline synthetase promoter, conferring tolerance to the antibiotic kanamycin (McCown et al., 1991).

The presence of the *bar* gene in the cranberry transformant displaying the best herbicide tolerance was confirmed by polymerase chain reaction (PCR) performed both at the University of Wisconsin (Michell Sass, personal communication) and at Agracetus Corp. (Mike Petersen, personal communication).

IN VITRO HERBICIDE TOLERANCE EVALUATION. Putatively transformed shoots were initially selected using kanamycin (Serres et al., 1992), as previous attempts to use phosphinothricin as the

selective agent were unsuccessful. Selected shoots were multiplied by cutting stem segments and the resulting microshoots were exposed to bialophos (the di-alanine conjugate of phosphinothricin) in an in vitro inhibition assay. Shoot tips 2 cm long were excised from actively growing shoot cultures and placed vertically to a depth of one node on a medium containing 0.25 mg·L^{-1} bialophos in 25×95 mm shell vials capped with Magenta two-way closures. A range of bialophos concentrations had been tested on untransformed shoots, and the 0.25 mg·L^{-1} level yielded sufficient growth inhibition to allow for selection without overwhelming the shoots. Increase in shoot length and root development were recorded after 5 weeks and a total of six transclones consisting of both 'Stevens' and 'Pilgrim' individuals was selected for further testing.

PLANT PROPAGATION AND GROWTH CONDITIONS. Plants were initially micropropagated (Serres et al., 1993). Microcuttings of transformed and untransformed cranberry were rooted in moistened commercial peat moss in covered plastic trays under continuous cool white fluorescent light (≈30 mmol·m^{-2}·s^{-1}) at 22 ºC. The trays were periodically misted with distilled water to keep the microcuttings moist. After 2 weeks, the plants were fertilized with 0.2 g·L^{-1} soluble fertilizer (Miracid, 30–10–10) and the trays were slowly vented to allow the plants to acclimate to ambient air. After another week, the plants were transferred to the greenhouse and maintained under 16-h photoperiod (with 200 mmol·m^{-2}·s^{-1} supplemental high-pressure sodium lighting) with 20 ºC minimum temperature. Thereafter the plants were fertilized weekly with 1.25 g·L^{-1} Miracid. After 6 to 7 weeks, these plants were used as stock plants and tip cuttings (≈10 cm long) were removed and rooted in moistened peat in $6 \times 6 \times 13$ cm plastic pots (tree bands) in a shaded fog tent in the greenhouse. The timing and handling were otherwise similar to that of micropropagated cuttings, and the resulting plants were equivalent. Plants for initial greenhouse treatments were ≈12 weeks old at the time of treatment.

For coldframe treatments, greenhouse plants were pruned thoroughly in late May or early June and placed outside in open frames. Fertilization was continued as above until terminated in mid-August. The age of the plants at treatment ranged from 16 to 18 weeks, depending on the timing of the treatment.

Plants of goldenrod (*Solidago* sp.) were originally collected from a young cranberry bed in central Wisconsin. For subsequent propagation, 5 cm rhizome tips with some roots attached were harvested from existing plants. These were placed in tree bands and otherwise handled the same as the cranberry plants, except that they were only pruned to remove excess growth. The age of these plants at treatment was variable, depending on the timing of cranberry treatments.

Plants of creeping sedge (*Carex chordorrhiza*) were originally collected from an established cranberry bed in northern Wisconsin. These were propagated by layering. Stolons extending from existing potted plants were bent and buried into new tree bands. When these had rooted, the stems to the parent plants were cut. These plants were otherwise handled the same as the goldenrod plants.

Untransformed control plants were either of the cultivar 'Stevens' or 'Pilgrim', depending on availability at the time of testing. Comparative herbicide spray treatments showed that the dosage response curve of these cultivars to foliar glufosinate applications was identical (data not shown).

INITIAL GREENHOUSE HERBICIDE TREATMENT. Plants of untransformed cranberry, the transclones, and goldenrod were used for each treatment of 0 or 100 mg·L^{-1} glufosinate (using the commercial formulation Liberty supplied by Agrevo, Wilmington,

Del.). Herbicide-free Liberty was added to the no-herbicide treatments to make the carrier level the same in both treatments. The plants were sprayed to runoff with a hand sprayer in the late afternoon. The plants were monitored for herbicide effects on growth, leaf chlorosis and leaf and shoot-tip necrosis over the following 4 weeks. Only one transclone was selected for further testing.

COLDFRAME HERBICIDE TREATMENTS OF TRANSFORMED PLANTS. Coldframe-grown plants of untransformed cranberry and the best performing transclone were sprayed in the early morning on rain-free days in midsummer. Plants were sprayed to runoff for each treatment. The first test evaluated glufosinate in 100 mg·L^{-1} increments up to 500 mg·L^{-1}. Subsequently, glufosinate concentrations up to 1000 mg·L^{-1} were also evaluated. All treatments, including controls, up to 500 mg·L^{-1} had a carrier concentration equivalent to 500 mg·L^{-1}, using herbicide-free Liberty to equalize any formulation differences. When higher herbicide levels were tested, the control treatment had a carrier level equivalent to the highest herbicide concentration being tested. The plants were visually evaluated for herbicide injury, and shoot-tip and whole plant survival were recorded. Goldenrod plants were included in all tests to insure that any herbicidal action observed was not unique to cranberry.

GENERATION AND HERBICIDE TREATMENT OF TRANSGENIC PROGENY. Plants of the selected transclone and untransformed plants of 'HyRed' (a high fruit color selection derived from a cross between 'Stevens' and a 'Ben Lear' selection) were allowed to stay in the coldframes until November and then transferred to an unheated greenhouse until January. The plants were then placed in a warm greenhouse under long days (as described above). Upon style emergence, the flowers of the transclone were self-pollinated; alternately, flowers of the transclone and 'HyRed' were emasculated prior to style emergence and reciprocal crosses were performed. Flowering, fruit and seed set, and fruit size of the transclone in the greenhouse were similar to that of untransformed 'Pilgrim'. The resulting first generation seedlings were germinated in vitro and otherwise grown and treated as described above, except that unreplicated individuals were established in 15 cm standard plastic pots instead of tree bands. These plants were sequentially treated every 2 weeks over the summer with increasing glufosinate levels from 100 to 1000 mg·L^{-1}. The plants were evaluated for herbicide injury and delay in growth. Those plants tolerating the highest glufosinate levels and able to set flower buds were flowered the following winter and self-pollinated as described above to generate an initial set of second generation of seedlings. The selected first generation plants were also cloned as described above and replicated testing for tolerance was performed the following summer at levels up to 8000 mg·L^{-1}.

The second generation seedlings were germinated and cloned in vitro to generate three replicated plants of each individual. These were established in tree bands and herbicide tolerance at 500 and 8000 mg·L^{-1} evaluated the next summer.

SOUTHERN ANALYSIS OF TRANSGENIC PROGENY. Total cellular DNA was isolated with a DNeasy Plant Maxi Kit (Qiagen, Valencia, Calif.) from greenhouse leaf tissue of untransformed 'Pilgrim', the original transclone and two selections from the first generation outcrossed progeny demonstrating high herbicide tolerance. DNA from each isolation was divided into two 10-µg aliquots and each aliquot digested with either *Hind*III (single cut site on the plasmid) or *Nco*I (two cut sites) restriction enzymes. Southern hybridizations were performed using procedures described by Sambrook et al. (1989), and detection as detailed by
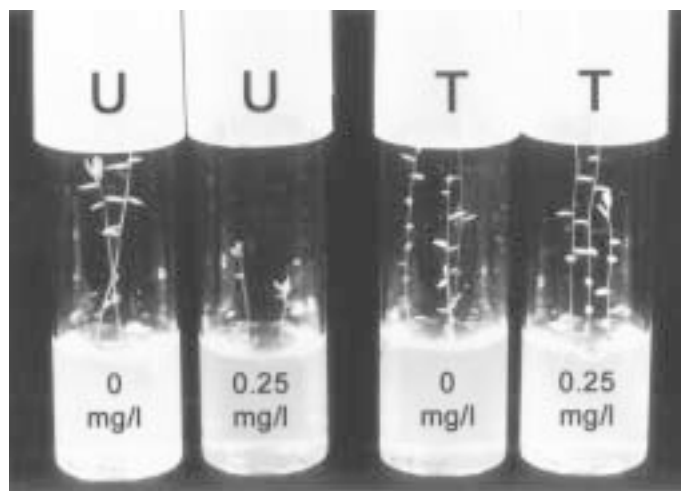
Fig. 1. Shoots of untransformed (U) and transformed (T) cranberry grown for 5 weeks in microculture with and without 0.25 mg·L^{-1} bialophos added to the medium.

Neuhaus-Url and Neuhaus (1993). Gels were stained with ethidium bromide to verify DNA presence following restriction digests before denaturation, blotting, and probe introduction. The hybridization probe (*bar* 512 bp) was nonradioactive DIGdATP-labeled using PCR. Each PCR reaction was 50 µL, consisting of
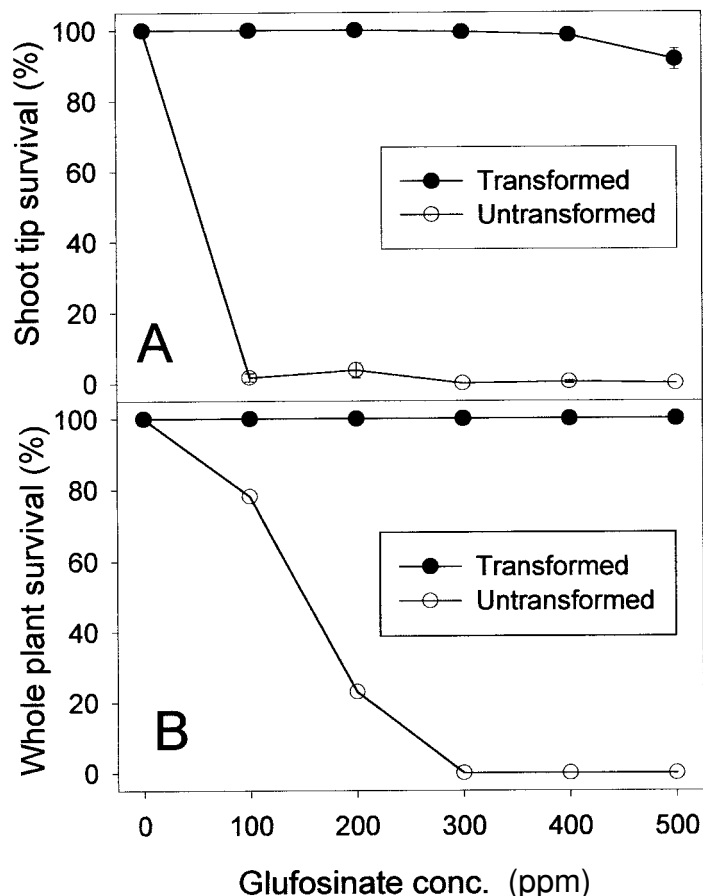

Fig. 2. The response of *bar*-transformed and untransformed cranberry plants to increasing concentrations of foliar sprays of glufosinate up to 500 mg·L^{-1}. Plants were grown in coldframes and treated in midsummer. (**A**) The percent of shoot tips surviving 2 weeks after treatment. Values are the mean ± SE of nine plants. (**B**) The percent of plants surviving after 4 weeks.

10× Expand buffer with MgCl$_2$, DIG probe synthetic mix, Expand Taq, 100 ng plasmid DNA and primers (forward primer nucleotides 4331 to 4354, GCAGGCTGAAGTCCAGCTGCCAG; reverse primer nucleotides 4843 to 4610, TGGTGAGCCCAGACCGACGCCCG).

STATISTICAL ANALYSIS. All plants used in a treatment series were grown in the same environment and were the same age. Plants were randomly assigned to treatment groups and during posttreatment evaluation, plants were randomized as to location on the bench or in the coldframe. Three replicate cranberry plants were included in each treatment in the initial greenhouse experiment and nine replicate cranberry plants were included in all other experiments, except as noted above for the screening of seedling plants. For shoot-tip survival, all tips on a plant were examined and an average survival calculated for each replicate plant. The number of shoot tips varied for each plant and ranged from 17 to 53 in the initial comparison between transformed and untransformed plants, and 10 to 16 for the comparison between the original transclone and its selected progeny. Chi-squared analyses of segregation were used to test goodness of fit.

Results and Discussion

IN VITRO HERBICIDE TOLERANCE. There was little indication of high levels of herbicide tolerance using the in vitro assays. Untransformed shoots were not killed after 5 weeks exposure to levels of up to 20 mg·L^{-1} bialophos in the medium. However rapid shoot growth (elongation) and the development of adventitious roots were strongly inhibited at levels of 0.1 mg·L^{-1} and above. The response of the putatively transformed regenerates was variable, however no regenerates grew well in a medium containing 1 mg·L^{-1} bialophos. The best regenerates grew and developed roots on a medium containing a low level of bialophos (0.25 mg·L^{-1}), a concentration that inhibited control shoots (Fig. 1).

The bialophos form of the herbicide was used for in vitro studies because it translocates more readily in shoots than the glufosinate form. Some studies have shown superior results using bialophos as the selective agent (Altpeter et al., 1996; Dennehey et al., 1994). Although direct selection using phosphinothricin agents has been successful for many crops, including woody perennials (Cabrera-Ponce et al., 1995; De Block, 1990), cases where such selection has not been effective also are evident (Bower et al., 1996; De Blondt et al., 1996).

INITIAL GREENHOUSE HERBICIDE TREATMENT. Cranberry plants treated with 100 mg·L^{-1} glufosinate immediately stopped growth and showed injury to the youngest leaves within a few days. Tip and young leaf necrosis was evident on all plants within 1 week and was followed by extensive necrosis throughout the exposed parts of the treated plants. Plants sprayed with only the carrier continued to grow normally and showed no effects.

The transclones displayed variable amounts of injury. The least injury was observed with one of the 'Pilgrim' transclones where the yellowing and necrosis was restricted to the upper 1 to 2 cm of the shoots. New growth from axillary buds developed after 1 week. By 4 weeks, all of the plants of this transclone were healthy and little evidence of herbicide injury persisted. All subsequent herbicide trials were conducted using only this 'Pilgrim' transclone (*bar*Pg) and its progeny.

No prominent herbicide injury occurred to the goldenrod plants at 100 mg·L^{-1} Liberty when grown in the greenhouse. A slight distortion of the rapidly growing shoot tips was occasionally observed, but further growth was normal.
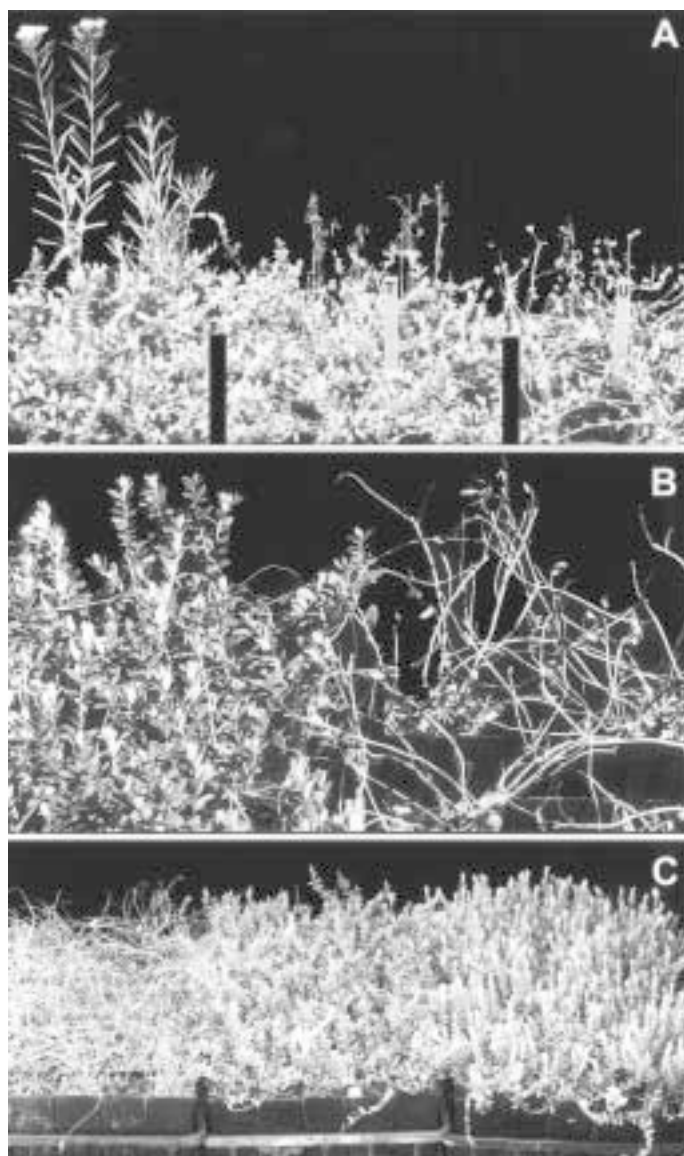
Fig. 3. Effect of glufosinate on *bar*-transformed and untransformed cranberry plants. **(A)** Three flats of coldframe-grown cranberry and goldenrod plants subjected to midsummer foliar sprays of glufosinate and photographed 2 weeks after treatment. The left flat contained carrier-only treated plants. The middle flat (T) contained cranberry plants transformed with the *bar* gene (*bar* 'Pilgrim') and sprayed with 500 mg·L⁻¹ glufosinate. The cranberry plants remained green while the goldenrod stems and leaves were killed. The right flat (U) contained untransformed cranberry plants sprayed with 500 mg·L⁻¹ glufosinate. All of these plants were killed or very severely injured. **(B)** Plants of transformed (**left**) and untransformed cranberry 4 weeks after spraying with 1000 mg·L⁻¹ glufosinate. While the transformed plants suffered some tip injury initially, all plants recovered. None of the untransformed plants survived. **(C)** Forty plant trays of untransformed (**left**), transformed (*bar* 'Pilgrim', center), and a highly tolerant 'HyRed' x *bar* 'Pilgrim' selection 3 weeks after a midsummer spray with 8000 mg·L⁻¹ glufosinate. Only the tips of the parent *bar* 'Pilgrim' were killed, whereas the untransformed plants were totally killed. The 'HyRed' x *bar* 'Pilgrim' selection showed no shoot tip death and only a brief delay in growth.

The initial conclusion from these greenhouse tests was that there was insufficient tolerance in the transformed plants to make this method of weed control commercially practical. Goldenrod grows similarly in both the greenhouse and the field. Cranberry, on the other hand, is far more succulent in the greenhouse, particularly under high nitrogen fertilization. Under field conditions, the growth is slower, the leaves smaller, and tissue less succulent. Sankula et al. (1997) reported that herbicide tolerance of *bar*-transformed rice was greater in more developed than less-developed (younger) plants, indicating that tissue quality is an important factor when evaluating glufosinate tolerance. Other researchers have found that evaluation of herbicide tolerance differs between field and greenhouse/laboratory conditions (D'Halluin et al., 1990; Garrod, 1989). Thus it was decided to further evaluate the herbicide tolerance under conditions more typical of cranberry field beds. The growth of cranberry plants grown in coldframes more closely resembles that of field-grown plants, and thus further evaluations were conducted in coldframes.

COLDFRAME HERBICIDE TREATMENTS OF TRANSFORMED PLANTS. Vegetative growth and vigor of the transformed and untransformed plants in the coldframe were equivalent, and were significantly less succulent than greenhouse plants. All transformed and untransformed coldframe plants from 500 mg·L⁻¹ equivalent carrier-only treatments continued to grow and showed no effects of the spray.

The initial coldframe trial revealed a greater differential in the herbicide effects between transformed and untransformed cranberry plants than that previously observed in the greenhouse. Levels as low as 100 mg·L⁻¹ glufosinate killed the vast majority of untransformed cranberry shoot tips within 2 weeks (Fig. 2A). Plant mortality at 4 weeks escalated with increasing herbicide concentrations up to 300 mg·L⁻¹, where none of the untransformed plants survived (Fig. 2B). Conversely, no plants of the transformed *bar*Pg were killed by any herbicide concentration tested (Figs. 2B and 3A), and shoot-tip survival was affected only above levels of 400 mg·L⁻¹ (Fig. 2A). There was a delay in growth of *bar*Pg plants up to 2 weeks and some irreversible injury to the young leaves at the higher herbicide levels. However, all these plants were healthy and actively growing by 4 weeks after spraying (Fig. 3A).

Injury to goldenrod plants also escalated with the level of herbicide treatment (data not shown). At 200 mg·L⁻¹, the leaf tips were injured, but the plants initiated new growth within 2 weeks. At 300 mg·L⁻¹, all shoot tips were killed, but new growth developed from the lower stems. At 400 mg·L⁻¹, most of the aboveground portions of the plants were killed. Carrier-only treated plants continued to grow and flower (Fig. 3A). The roots and rhizomes of goldenrod were not killed by any herbicide level. Sedge plants grown in the coldframes were injured by 100 mg·L⁻¹ and were capable of regrowing from basal parts; 400 mg·L⁻¹ treatments killed sedge plants.

The above results confirmed that these coldframe transgenic plants were considerably more tolerant to glufosinate than shoots grown under greenhouse conditions. To further determine the

Table 1. Chi squared analysis of herbicide tolerance segregation in selfed and outcrossed transgenic cranberry progeny.

Cross	Progeny tested	Tolerant progeny	Susceptible progeny	Ratio tested	χ^2 value
bar 'Pilgrim' x self	88	50	38	3:1	15.52[**]
bar 'Pilgrim' x 'HyRed'	94	50	44	1:1	0.38[NS]
'HyRed' x *bar* 'Pilgrim'	52	21	31	1:1	1.92[NS]

[NS],[**]Nonsignificant or significant at $P < 0.01$, respectively.

level of tolerance achieved, a second test with higher glufosinate concentrations (up to 1000 mg·L⁻¹) was performed. While there was some increase in shoot tip injury, all the transformed plants survived and had resumed active growth by 4 weeks (Fig. 3B), while the herbicide-treated untransformed plants were killed.

COLDFRAME HERBICIDE TREATMENTS OF *bar*Pg PROGENY. The *bar* gene was successfully transferred to both self-pollinated and outcrossed progeny, based on the ability to tolerate at least 100 mg·L⁻¹ glufosinate. Cranberry is a diploid species, and when *bar*Pg was crossed to the untransformed 'HyRed', the number of tolerant to susceptible progeny agreed with the expected 1:1 ratio for a single insertion site (Table 1). However, the selfed population was below the expected 3:1 ratio. Most of the tolerant progeny demonstrated a level of tolerance similar to that of the *bar*Pg parent, based on sprays with escalating glufosinate concentration. Some outcrossed individuals were more tolerant than the *bar*Pg parent, as they showed no tip death or delay in growth at 1000 mg·L⁻¹ glufosinate. For further evaluation, replicated plants were initially screened in the greenhouse (data not shown) and two individuals from the outcrossed progeny (one 'HyRed' x *bar*Pg and one *bar*Pg x 'HyRed') were selected for replicated testing in the coldframe. The two selected progeny were able to tolerate high levels of glufosinate, up to 4000 mg·L⁻¹, without significant shoot tip necrosis (Fig. 4). A subsequent bulk test using groups of forty plants confirmed the ability of one of these selections to tolerate 8000 mg·L⁻¹ with only minimal delay in growth (Fig. 3C).

One explanation for the elevated herbicide tolerance in some of the progeny is a reduction in transgene copy number or other rearrangements in the inserted genes that would favor enhanced gene expression. For further evaluation, Southern analysis was performed on these individuals. The results indicated that multiple copies of the *bar* gene were stably inserted, as a band equivalent in size to the whole plasmid (5511 base pairs) was recovered with *Hind*III digestion, in addition to two smaller bands (likely fragments produced from the outlying plasmid ends to restriction sites within the plant genome) (Fig. 5). Similar results were obtained in a previous Southern probing for CaMV 35s in *bar*Pg using *Hind*III, *Xba*I, and *Bgl*II digests (all single restriction sites on the plasmid, data not shown). The *Nco*I

digestion (Fig. 5) supports multiple copies as well since the expected plasmid-only fragments (≈3700 and 1800 base pairs) were observed, with two additional bands like the others. Because only two nonplasmid bands were produced in each digest, the Southerns indicate a single insertion site. There was no difference in the banding pattern between the higher tolerant selections and the *bar*Pg parent, suggesting that the difference in tolerance was not due to rearrangement of the inserted genes. As expected, untransformed 'Pilgrim' showed no hybridization signal.

Two other explanations for enhanced herbicide tolerance in the selected progeny are an interaction with other factors in the native genome (such as elevated glutamine synthetase levels) or a repositioning of the inserted genes to a higher expressing area. Analysis of the phospinothricin acetyl transferase and glutamine synthetase enzyme activities would be required to begin to address such hypotheses. However, a clear explanation for the observed enhanced tolerance in the progeny is not obvious.

INITIAL EVALUATION OF SECOND GENERATION PROGENY. While the majority of the *bar*Pg selfed progeny were tolerant, the number of tolerant individuals was less than expected. Gene silencing may be one explanation. A variety of plant systems have shown significant levels of transgene silencing (Iyer et al., 2000; Kumar and Fladung, 2001; Morel et al., 2000). In lettuce, increasing levels of transgene silencing were observed with successive generations when the CaMV 35s promoter was used (McCabe et al., 1999). In our studies with cranberry, a limited test of 39 second generation seedlings (derived from self-pollinating the two highly herbicide-tolerant first generation progeny selections) was conducted using replicated testing at 500 mg·L⁻¹. No indica-
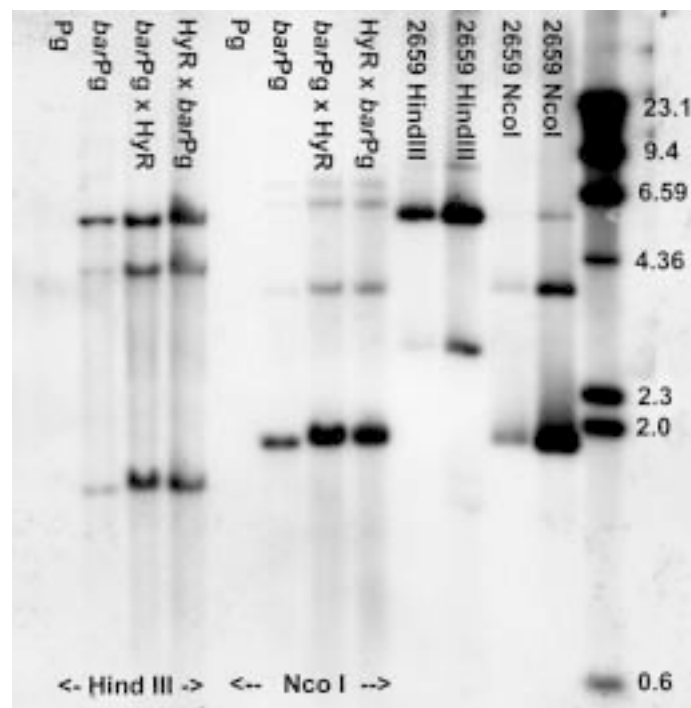
Fig. 5. Autoradiogram from hybridization of *bar* to untransformed 'Pilgrim' cranberry (Pg), *bar*-transformed 'Pilgrim' (*bar*Pg) and two selections from outcrossed progeny with high herbicide tolerance (one *bar*Pg x 'HyRed' and one 'HyRed' x *bar*Pg). The DNA from these samples was cut with two restriction enzymes, *Hind*III (left) or *Nco*I (center) and compared to the original source plasmid (2659, *Hind*III or *Nco*I as indicated). Molecular weight markers are indicated on the right. The fragments suggest the presence of multiple copies of the *bar* gene at a single site in the original transformant and no rearrangements in the selected progeny.
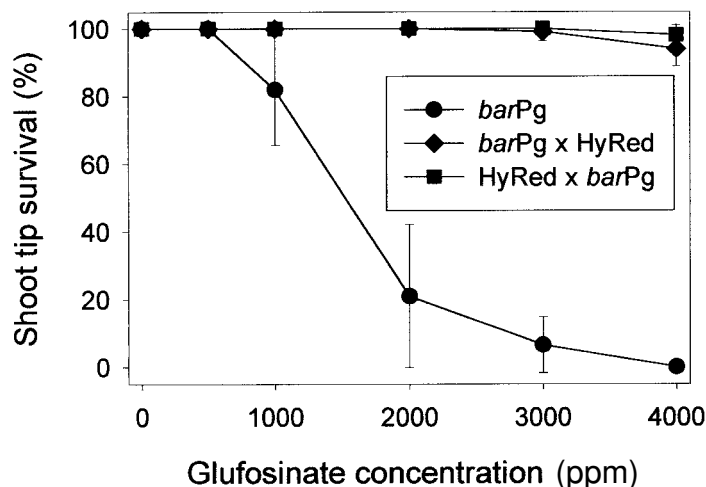

Fig. 4. The survival of shoot tips of transformed 'Pilgrim' cranberry (*bar*Pg) and two selected progeny (one *bar* 'Pilgrim' x 'HyRed' and one 'HyRed' x *bar* 'Pilgrim') to increasing concentrations of foliar sprays of glufosinate up to 4000 mg·L⁻¹. Plants were grown and tested in coldframes in midsummer. Values are the mean ± SE of nine plants.

tion of gene silencing was observed in this population, as a ratio close to the expected 3:1 tolerant to susceptible was observed (25 tolerant and 9 susceptible). In addition, when tested at 8000 mg·L^{-1}, a majority (17) of the tolerant second generation progeny displayed the high tolerance (no shoot tip necrosis) of their parents.

STRATEGIES FOR THE USE OF HERBICIDE TOLERANT CRANBERRIES. These results demonstrate the feasibility of successfully transforming cranberry for the expression of an introduced herbicide tolerance gene. Expression of the introduced gene in coldframe plants has been stable over multiple seasons after the initial transformation. The introduced trait has been successfully transferred to two generations of progeny, with enhanced levels of tolerance evident in selected outcrossed progeny.

The definitive timing and dosage of herbicide applications will eventually have to be established under actual field conditions. An important time for cranberry growers to control weeds is early in the life of a bed, when cranberry vines are becoming established and are more susceptible to competition from weeds. Established and well-maintained cranberry beds are very competitive and thus invasion by weeds is slow. The use of an herbicide such as Liberty may be most intensive during the first 5 years after planting a bed. After establishment, only periodic use in specific weed-infested areas would be warranted, thus helping to avoid residue and weed resistance problems. Any resistant weeds that might develop could still be controlled using glyphosate wiping, which the growers currently use.

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