Many clonal plants consist of many connected individual ramets, allowing them to share water and nutrients via physiological integration. Integration among ramets may also improve the ability of clonal plants to tolerate abiotic stress or improve the competitive ability of individual ramets. Here I use a field experiment to determine whether clonal integration improves ramet performance for a widespread clonal tree species invading into native prairie. Aspen (*Populus tremuloides*) dominates the southern treeline in western Canada, has long-lived belowground connections between mother and daughter ramets, and reproduces vegetatively via resprouting rhizomes after disturbance. I applied two competition treatments (neighbors present or absent) and two clonal integration treatments (belowground rhizomes between mother and daughter ramets either severed or left intact) to 12 replicate *Populus* daughter ramets at each of three sites. Neighbors improved the survivorship of *Populus* ramets by 25–35% after 2 yr, but decreased growth by ~20%. Clonal integration tended to improve ramet survival and growth, but these trends were often not significant. Clonal integration did not alter the effects of competition from neighboring vegetation, suggesting that connections between ramets do not necessarily improve the competitive ability of *Populus* invading into native prairie.

**Key words:** clonal plant; competition; grassland; physiological integration; *Populus tremuloides*; prairie; tree–grass competition; tree invasion.

Physiological integration enables clonal plants to transfer water, carbohydrates, and mineral nutrients among ramets (Alpert and Mooney, 1986; Stuefer and Hutchings, 1994; Alpert, 1996; Wijesinghe and Hutchings, 1997), exploit patchy resources (de Kroon and Knops, 1990; Evans and Cain, 1995; Brewer and Bertness, 1996), and ameliorate environmental stresses (Salzman, 1985; Salzman and Parker, 1985; Slade and Hutchings, 1987; Pennings and Callaway, 2000), possibly improving the fitness of clonal plants (see reviews by Pitelka and Ashmun, 1985; de Kroon and van Groenendael, 1997). For example, resource exploitation and sharing among ramets of clonal plants often enhance the survival and decrease the effects of competition for individual ramets (Hartnett and Bazzaz, 1985; Evans, 1988, 1992; Hester et al., 1994; Brewer and Bertness, 1996). Recent studies in natural vegetation have demonstrated that physiological integration does not necessarily improve the competitive ability of clonal plants (Amsberry et al., 2000; Pennings and Callaway, 2000). Here, I determine whether physiological integration enhances ramet survival, growth, and response to interspecific interactions of a clonal tree invading prairie.

The invasion of grassland by woody plants is widespread over many parts of the world (reviewed by Archer et al., 1988; Archer, 1995; Wilson, 1998). Here a field experiment is used to determine whether clonal integration improves the survival and growth of aspen (*Populus tremuloides* Michx.), a common, widespread clonal tree species, invading into native mixed-grass prairie. To determine whether clonal integration in *Populus* improves the performance of individual ramets, I asked the following questions: (1) Do ramets connected to the mother clone have higher survivorship than ramets severed from the mother clone (i.e., without physiological integration)? (2) Are connected ramets less suppressed by competition from prairie vegetation than severed ramets? Greater performance of connected ramets is evidence for the beneficial effects of physiological integration in *Populus*. One benefit of subsidizing new daughter ramets on the edge of the mother clone may be the ability to establish ramets in the neighboring vegetation thereby facilitating invasion and displacing neighbors. This same mechanism would also allow colonization of adjacent habitats or persistence in altered habitats in which seed establishment is uncommon.

**MATERIALS AND METHODS**

**Study site—**I worked at White Butte Recreation Area (50°28′ N, 104°22′ W), 18 km east of Regina, Saskatchewan, Canada. The vegetation comprises discrete patches of aspen forest (*Populus tremuloides* Michx.) with a snowberry (*Symphoricarpos occidentalis* Hook.), raspberry (*Rubus idaeus* L. var. *aculeatissimus* Regel & Tiling), or meadowssweet (*Spirea alba* Du Roi) understory and mixed-grass prairie dominated by *Agropyron spp.*, *Bouteloua gracilis* (HBK) Lag., *Corylus spp.*, *Koelaria macrantha* (Ledeb.) J. A. Schultes f., *Poa spp.*, *Sipta comata* Trin. & Rupr., and *Selaginella densa* Rydb (nomenclature follows Looman and Best 1987) (further background on this site is provided by Wilson [1993], Kleb and Wilson [1997], Köchy and Wilson [1997]. The climate is continental with mean daily temperatures of −17°C in January and 19°C in July (Environment Canada 1993). The mean annual precipitation is 384 mm, mainly falling from May to September. Parent soils for both forest and prairie vegetation at White Butte are regosols on silty sand (Agriculture Canada, 1992).

*Populus* is a widespread, clonal tree that forms monospecific stands (at the tree layer) within a prairie matrix in the study area (Peterson and Peterson, 1992; Peterson and Jones, 1997). *Populus* is a shade-intolerant species with strong apical dominance, characteristics that favor ramet initiation along clone edges or after disturbance (e.g., following fire, hail, or wind damage that removes apical dominance) and discourage the production of ramets in shad-
ed, adult clones (Bailey and Wroe, 1974; Bailey, Irving, and Fitzgerald, 1990; Peterson and Jones, 1997; Peltzer et al., 2000). *Populus* has long-lived belowground connections between individual stems or ramets and usually reproduces vegetatively via rhizomes (Zasada, Shariik, and Nygren, 1985; Krasny and Johnson, 1992; Peterson and Peterson, 1992). Belowground connections between ramets of *Populus* are known to transfer dye, herbicide, and ^42^Ru up to 13 m between ramets (DeBylle, 1964; Peterson and Peterson, 1992). In addition, the roots of dead ramets (trees) may remain alive for several years, presumably supported by photoassimilate from neighboring healthy ramets.

In 1996, a severe hailstorm damaged many mature *Populus* stems in the study area resulting in a wave of vegetative reproduction in early 1997 (D. A. Peltzer, unpublished data). Daughter ramets extend ~10–25 m away from the edge of mature aspen clones into the surrounding native mixed-grass prairie. Competition and clonal integration treatments were applied to these daughter ramets in order to test the hypotheses that clonal integration improves plant performance and competitive ability.

**Experimental treatments**—To assess the effects of clonal integration and competition on the performance of *Populus* invading into prairie, *Populus* ramets were selected at the forest–prairie boundary at three sites (each separated by ~300 m). Ramets (hereafter termed daughter ramets) were randomly selected 5–10 m from the edge of mature *Populus* stands (hereafter called mother clones) with a minimum spacing of 5 m between ramets, tagged, measured for initial size in early May 1998, and their condition (i.e., alive or dead) was recorded in spring and fall, 1998 and 1999.

Two competition treatments were used to examine the effects of prairie vegetation on daughter ramet performance. Ramets were grown either with neighboring vegetation removed (no neighbors, NN), or with neighbors left intact (all neighbors, AN). For the NN competition treatment, neighbors were sprayed with a nonselective herbicide (3% glyphosate solution, trade name “RoundUp”; Monsanto, St. Louis, Missouri, USA) in early May 1998. Ramets were protected from herbicide by covering the stems with thin plastic during herbicide application. Stems of woody neighbors were clipped at the soil surface in a 0.8 m radius around each ramet. Belowground competition was eliminated by trenching to a depth of 35 cm in a 0.8 m radius around each ramet; this is the rooting zone containing most roots of both woody and herbaceous species in this system (see Wilson 1993, Peltzer 2001). Care was taken not to disrupt the belowground connection between the daughter ramet and mother clone (see below). For the AN competition treatment, surrounding plants were neither sprayed nor clipped. Roots were trenched in the AN treatment to control for the disturbance caused by trenching, but the clonal connection between ramet and maternal clone was located (see below), flagged, and left intact.

To assess the role of physiological integration in the performance of *Populus*, the belowground rhizome connecting daughter ramets to their mother clone was either left intact or severed. Severing rhizome connections mimics natural losses of clonal connections by belowground herbivores, physical damage, or disease. Although severing connections is a standard approach to studying the importance of clonal integration, it may cause trauma or introduce disease (Kelly, 1995). Several studies have found no effect of severing connections in control conditions, suggesting that the severing treatment is not necessarily severe (Hartnett and Bazzaz, 1983; Alpert, 1991; Evans, 1992; Hester et al., 1994; Pennings and Callaway, 2000). I did not observe any sudden death or increased disease of severed ramets that would suggest the severing treatment directly affected ramets.

Belowground clonal connections were typically located 2–10 cm below the soil surface. The location of rhizomes was assessed using thin stainless steel probes (1 mm diameter), which were inserted into the soil at the base of each daughter ramet and used to probe and follow the rhizome to the mother clone. To assess the location of clonal connections and the efficacy of these treatments, several preliminary attempts (*N* = 10) were made on randomly selected ramets. The location of belowground connections was assessed using probes to locate rhizomes prior to root trenching or severing clonal connections. All trial ramets were excavated. These trials confirmed that this technique was effective.

In summary, the experimental design included two competition treatments and two severing (clonal integration) treatments. Competition and severing treatments were fully interspersed among daughter ramets. The entire experiment was repeated at three sites using 12 replicate daughter ramets of *Populus* for each treatment combination.

**Ramet performance**—All *Populus* ramets were tagged and randomly assigned to competition and severing treatments. Initial ramet mass was measured according to the following allometric equation developed from 30 ramets collected from the study sites representing the full range of ramet sizes (*M* = *a* + *bx*, *r*² = 0.93, *P* < 0.001, *N* = 30) where *M* = aboveground biomass (in grams), *x* = the log-transformed stem diameter at 10 cm height (in millimeters), and *a* and *b* are fitted parameters. The performance of *Populus* ramets was assessed as survival and growth, recorded in May and September 1998 and 1999. Growth was calculated as relative growth rate (RGR): 

\[ RGR = \frac{\ln(M_f) - \ln(M_i)}{d} \]

where *M*<sub>f</sub> is the final shoot mass, *M*<sub>i</sub> is the initial shoot mass, and *d* is the number of days between biomass measurements calculated separately for summer 1998, winter 1998, and summer 1999. Dead ramets were not included in the analysis of growth. Competition intensity (CI), the proportional reduction in ramet growth caused by neighbors, was calculated as: 

\[ CI = \frac{RGR_{NN} - RGR_{AN}}{RGR_{NN}} \]

where RGR<sub>NN</sub> and RGR<sub>AN</sub> are the growth rates for ramets in NN and AN competition treatments, respectively (Wilson and Keddy, 1986). Relative measures of competition intensity are preferred over absolute reductions in plant survival or growth (see discussions by Goldberg, 1994; Grace, 1995).

**Resource availability and neighbors**—Resource levels were measured over the growing season to determine the effects of experimental treatments on resource availability. Soil moisture and light availability were measured in late July 1998 and 1999, the period of peak standing crop. Three soil cores (2 cm diameter, 15 cm deep) were collected within 20 cm of each ramet and pooled. Soil moisture was determined gravimetrically for each pooled sample.

Light penetration was measured in late July in a cloudless day within 1 h of solar noon using a 1 × 40 cm integrating light probe (Sunfleck ceptometer, Decagon, Pullman, Washington, USA). One measurement was made 10 cm above the vegetation or ramet, and two measurements were made perpendicular to one another at the soil surface beneath each ramet. Light penetration to the soil surface was calculated as the proportion of light above the vegetation.

In 1998, the aboveground biomass of vegetation surrounding each ramet was measured. For woody plants, all stems were counted and measured in a 50 cm radius around each ramet. Biomass of each woody species was calculated according to allometric equations developed at the site for each species of woody plant (D. A. Peltzer, unpublished data). Herbaceous plants were clipped at ground level in a 10 × 50 cm quadrant beside each ramet, dried (70°C, 2 d), and weighed.

**Data analysis**—Ramet survivorship at the end of each growing season was tested among treatments and their interactions with likelihood ratio chi-square tests using logistic regression (JMP version 3.2, SAS, 1997). Differences in growth and resource availability between competition and clonal connection treatments were examined using repeated measures ANOVA (Stevens, 1992; vonEnde, 1993). There were no differences between results from univariate repeated measures ANOVA and MANOVA (Greenhouse-Geisser and Huynh-Feldt adjustments did not affect probability levels because epsilon approached 1 for both methods); only results from MANOVA are presented. Competition and clonal connection were treated as fixed effects, and site was treated as a random factor for this analysis. Log transformations prior to data analysis improved the normality and homoscedasticity of the data (Zar, 1984). A significant interaction between competition and clonal connection would suggest that physiological integration modifies the effects of competition on *Populus* growth. A significant three-way interaction among time, competition, and clonal connection would suggest that physiological integration modifies the effects of competition differently between years. Linear regression was used to determine the dependence of growth on neighborhood biomass after log transformation of these variables.
RESULTS

Survivorship—Survivorship of *Populus* daughter ramets was significantly higher with neighbors present (AN competition treatment) than without neighbors (NN) (Fig. 1; logistic regression: main effect of competition [C]: likelihood ratio [LR]χ² = 16.1, \( P < 0.001 \)). In contrast, severing clonal connections did not influence survival (severing effect [S]: LR χ² = 1.8, \( P = 0.18 \)) or alter the effects of neighbors on ramet survival (C × S: LR χ² = 23.2, \( P = 0.13 \)).

*Populus* survivorship decreased from ~90% in 1998 to 65–75% with neighbors present or 40% without neighbors by late 1999 (Fig. 1; Time: LR χ² = 36.4, \( P < 0.001 \); C × T: LR χ² = 23.1, \( P < 0.001 \)). Clonal integration increased survivorship ~10% by late 1999, resulting in a significant Time × Severing interaction (χ² = 5.80, \( P = 0.016 \)).

Growth—Neighbors reduced ramet growth by ~20% in both 1998 and 1999 (AN vs. NN in Fig. 2; Table 1). Severing significantly decreased growth, but did not alter the effects of competition (Table 1).

Growth was generally 0.005 \( g \cdot g^{-1} \cdot d^{-1} \) in 1998, increased over the winter of 1998/1999, and was approximately fourfold higher in 1999 (Fig. 2); however, the effects of competition and clonal integration on growth did not vary through time (Table 1).

The growth of daughter ramets was significantly reduced by neighbors, whether ramets were severed or not from the mother clone (ANOVA intercept for CI = 0.22, \( t_i = 21.8, \ P = 0.029 \); Severing: \( F_{1,2} = 2.28, \ P = 0.270 \)). Growth did not significantly decline with aboveground neighbor biomass over the observed range of 54.9–2745 g/m² (linear regression of log-transformed 1998 growth vs. log aboveground neighbor biomass: intercept = −6.04, slope = −0.018, \( r^2 = 0.01 \), \( P = 0.93, N = 72 \)).

Resource availability—Mean soil moisture (±1 SE) was 5.31% (±0.27%) with neighbors and 5.12% (±0.25%) without neighbors and did not vary significantly among any treatment combinations (ANOVA: Competition: \( F_{1,137} = 0.55, \ P = 0.460 \); Severing: \( F_{1,137} = 0.61, \ P = 0.436 \); C × S: \( F_{1,137} = 0.36, \ P = 0.550 \); data are for 1998; results are similar for 1999). Light penetration was much lower in AN competition treatments (17.3 ± 1.7%) than in NN treatments (76.1 ± 2.8%), but did not vary significantly among other treatment combinations (Competition: \( F_{1,137} = 191.4, \ P < 0.001 \); Severing: \( F_{1,137} = 0.87, \ P = 0.353 \); C × S: \( F_{1,137} = 1.03, \ P = 0.312 \); 1998 data).

DISCUSSION

Several lines of evidence suggest that clonal integration influenced the performance of new *Populus* ramets in prairie, but that integration did not alter the effects of competition on ramets. Severing clonal connections between mother and daughter ramets did not significantly alter *Populus* survival (Fig. 1), but did reduce growth by 20–30% (Fig. 2). No significant interactions between severing and competition or time were observed, suggesting that physiological integration did not modify the effects of competition or vary through time. In contrast, both competition and time had strong influences on ramet performance. Neighbors improved survivorship by 25–35%, perhaps through shading or facilitation, but also decreased growth by 20% (Figs. 1 and 2). Ramet survivorship declined to 40–80% after 2 yr whereas growth increased three-to fourfold over the same period. Taken together, these results suggest that both competition and ramet age (time) more strongly regulate ramet performance than does clonal integration.
Several previous studies have also observed that physiological integration does not necessarily improve the competitive ability of clonal plants, at least in some environments (Schmid and Bazzaz, 1987; Williams and Briske, 1991; de Kroon, Hara, and Kwant, 1992; de Kroon, 1993; Hartnett, 1993; de Kroon and Knops, 1990; Pennings and Callaway, 2000; but see Hartnett and Bazzaz, 1985). In this study, the intensity of competition (measured as the proportional reduction in ramet growth) experienced by *Populus* ramets was similar whether belowground connections between mother and daughter ramets were intact or severed (Fig. 2). Growth of *Populus* in intact vegetation tended to be slower with clonal connections severed, but this effect was not significant; similar results were observed for severed daughter ramets of six clonal salt marsh plant species (Pennings and Callaway, 2000). Severing clonal connections had no significant effects on intra- or interspecific competition for three species of *Aster* or *Solidago gigantea* growing in an old field (Schmid and Bazzaz, 1987) or on intraspecific competition in *Brachypodium pinnatum* or *Carex flacca* (de Kroon, Hara, and Kwant, 1992). In contrast, severing clonal connections in *Solidago canadensis* reduced its competitive ability against interspecific neighbors (Hartnett and Bazzaz, 1985). The importance of clonal integration in interspecific interactions clearly deserves further study.

Clonal integration may be more important for exploiting patchy resources or tolerating stressful environments than improving competitive ability. In support of this idea, Pennings and Callaway (2000) studied the role of physiological integration in six species of clonal salt marsh plants and observed that clonal integration was very important for plants invading salt pans (stressful microhabitat), moderately important for plants invading plots with neighbors clipped, and least important for plants invading into intact vegetation. Cain (1994) reported that intraspecific neighbors did not reduce the survival or size of daughter ramets in the rhizomatous perennial herb *Solidago altissima* in several New York old fields. These observations are consistent with other studies showing that reallocating resources to daughter ramets may allow clonal plants to rapidly exploit resource patches (Salzman, 1985; de Kroon and Knops, 1990; Cain, 1994; Evans and Cain, 1995; Shumway, 1995; Brewer and Bertness, 1996; Stuefer, de Kroon, and During, 1996; Stuefer, During, and de Kroon, 1996; Stoll, Egli, and Shmid, 1998), but does not support the hypothesis that physiological integration improves ramet competitive ability. These studies suggest that physiological integration may be most important in stressful or low-productivity environments, but further work is needed to understand how clonal connections affect plant performance in different environments (e.g., Loehle and Jones, 1987; Grace, 1993; Jönsdóttir and Watson, 1997).

Daughter ramet survival ranged from 40 to 80% after two growing seasons (Fig. 1), comparing well with 62% *Populus*

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**Table 1.** Repeated-measures ANOVA results for the influence of competition (C), severing clonal connections between mother and daughter ramets (S) and site (Site) and Time (T) on the growth of *Populus tremuloides* ramets in 1998 and 1999 (Fig. 2). Competition and clonal connection treatments were applied within three replicate sites.

<table>
<thead>
<tr>
<th>Source</th>
<th>df*</th>
<th>Wilks’ lambda</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All between Site</td>
<td>5, 28</td>
<td>0.8731</td>
<td>0.814</td>
<td>0.550</td>
</tr>
<tr>
<td>C</td>
<td>2, 28</td>
<td>0.9998</td>
<td>0.004</td>
<td>0.997</td>
</tr>
<tr>
<td>S</td>
<td>1, 28</td>
<td>0.3050</td>
<td>4.94</td>
<td>0.002</td>
</tr>
<tr>
<td>C × S</td>
<td>1, 28</td>
<td>0.2259</td>
<td>5.70</td>
<td>0.024</td>
</tr>
<tr>
<td>All within Site</td>
<td>10, 54</td>
<td>0.7690</td>
<td>0.758</td>
<td>0.668</td>
</tr>
<tr>
<td>C × T</td>
<td>2, 27</td>
<td>0.9012</td>
<td>1.48</td>
<td>0.246</td>
</tr>
<tr>
<td>S × T</td>
<td>2, 27</td>
<td>0.9288</td>
<td>1.04</td>
<td>0.369</td>
</tr>
<tr>
<td>C × S × T</td>
<td>2, 27</td>
<td>0.9040</td>
<td>1.43</td>
<td>0.256</td>
</tr>
</tbody>
</table>

* Numerator, denominator df for MANOVA F.
survival 3–4 yr after clear-cutting forest in Arizona (Jones and DeByle, 1985), 37–62% 2 yr after forestry site preparation or wildfire in central Saskatchewan (D. A. Peltzer, unpublished data), and 7% survival 12 yr after disturbance in New York clearcuts (Krasny and Johnson, 1992). Perhaps strong self-thinning in *Populus* and other clonal plants occurs because of weak physiological integration among ramets, but this possibility remains to be tested (see also discussion by de Kroon, 1993).

Several other factors not examined in the current study may account for the observed variation in performance among *Populus* ramets. Daughter ramets may be moving resources, e.g., water and nutrients, to the mother ramet. Bidirectional movement of water, nutrients, or photosynthate between daughter and mother ramets can be evaluated using stable isotopes or tracers (e.g., de Kroon et al., 1996). These techniques could be used to evaluate whether there is increased resource allocation from daughter ramets to mother ramets when neighboring vegetation is removed. Here, light availability and soil moisture could not be used to predict daughter ramet performance, suggesting that these resources were not limiting in this system or that other environmental or biotic factors more strongly regulated ramet performance. These topics certainly deserve further consideration.

In summary, neighbors increased the survival but decreased the growth of *Populus* ramets. In contrast, clonal integration did not generally alter ramet performance or the effects of competition, suggesting that interspecific interactions may be more important than physiological integration in this system. Further work is needed to compare the ecological benefits of clonal integration among species and environments to better understand the role of integration in the field.

**LITERATURE CITED**


SAS. 1997. JMP version 3.2. SAS Institute, Cary, North Carolina, USA.


