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Short Title: Transgressive segregation in fleabane

Transgressive segregation and the inheritance of paraquat resistance in Canada fleabane (*Conyza canadensis*)

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Abstract

Transgressive segregation refers to the phenomenon whereby the progeny of a diverse cross exhibit phenotypes that fall outside the range of the parents for a particular trait of interest. Segregants that exceed the parental values in life history traits contributing to survival and reproduction may represent beneficial new allelic combinations that are fitter than respective parental genotypes. In this research, we use geographically disparate paraquat resistant biotypes of horseweed (Canada fleabane) [Conyza canadensis (L.) Cronquist; syn. *Erigeron canadensis* L.] to explore transgressive segregation in biomass accumulation and the inheritance of the paraquat resistance trait in this highly self-fertilizing species. Results of this research indicated that the paraquat resistance traits in *E. canadensis* biotypes originating in California, USA and Ontario, Canada were not conferred by single major gene mechanisms. Segregating generations from crosses among resistant and susceptible biotypes all displayed transgressive segregation in biomass accumulation in the absence of the original selective agent, paraquat. However, when challenged with a discriminating dose of paraquat, progeny from the crosses of susceptible x resistant and resistant x resistant biotypes displayed contrasting responses with those arising from the cross of two resistant biotypes no longer displaying transgressive segregation. These results support the prediction that transgressive segregation is frequently expressed in self-fertilizing lineages and is positively correlated with the genetic diversity of the parental genotypes. When exposed to a new environment, transgressive segregation was observed regardless of parental identity or history. However, if hybrid progenies were returned to the parental environment with exposure to paraquat, the identity of fittest genotype (i.e., parent or segregant) depends on the history of directional selection in the parental lineages and the dose to which the hybrid progeny was exposed. It is only in the original selective environment that the impact of allelic fixation on transgressive segregation can be observed.

Key Words:, fitness, herbicide resistance, hybridization, transgressive segregation,

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Introduction

Transgressive segregation refers to the phenomenon whereby the progeny of a diverse cross exhibit phenotypes that fall outside the range of the parents for a particular trait of interest (Mackay et al. 2021). At the genetic level, transgressive segregation has often been ascribed to the dispersal of favourable alleles from the parents of a cross to its progeny (de Los Reyes 2019; Mackay et al. 2021; Rieseberg et al. 1999). While transgressive segregation is often mentioned alongside the more widely discussed phenomenon of heterosis, they are differentiated by the fact that heterosis is most evident in the F_1 generation and, by definition, must also show directional dominance (Mackay et al. 2021). In contrast, transgressive segregation is predominantly expressed in the F_2 generation, with segregants that transcend the parental mean in either direction. Plant breeders have long taken advantage of transgressive segregation to select improved cultivars with many studies reporting transgressive segregants for agronomically important traits of interest, including seed oil content (Alt et al. 2005), pathogen or disease resistance (Winter et al. 2007) and grain yield (Vega and Frey 1980).

In their review of transgressive segregation, Rieseberg et al. (1999) outlined several instances where we would expect to see transgressive segregation frequently expressed. Based on the assumption that transgressive segregation is underpinned by complementary allelic action, the authors predicted that we should expect it to be most frequently observed in crosses between individuals from self-fertilized species and positively correlated with the genetic divergence of the parental biotypes. The authors also predicted that traits with a history of directional selection are less likely to exhibit transgressive segregation when compared to those that have undergone genetic drift or stabilizing selection. Since its publication a number of studies have explored these predictions as a framework for understanding how transgressive segregation might influence the processes of adaptation and speciation in a range of natural ecosystems (Bell and Travis 2005; Lamichhaney et al. 2018).

With the notable exception of plant breeders and geneticists, transgressive segregation has received comparably little attention in the agricultural literature. The field of weed science in particular would benefit from a deeper understanding of transgressive segregation and its implications for weed management, particularly with respect to crop-weed hybridization, invasive species and the spread of herbicide resistance among and within populations (Campbell

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et al. 2006; Clements and Jones 2021; Jasieniuk et al. 1996). Of the few weed science studies where transgressive segregation has been explicitly discussed (Giacomini et al. 2019; Liu et al. 2019; Zelaya et al. 2007), a study of its impact on fitness in slender wild oat (*Avena barbata* Pott ex Link) is perhaps the most detailed (Johansen-Morris and Latta 2006). Through their study of this highly self-fertilizing species, Johansen-Morris and Latta (2006) demonstrated that single hybridization events between genetically divergent biotypes can result in a range of potential outcomes for the progeny, including hybrid vigor, hybrid breakdown and transgressive segregation. Importantly, the results of this study demonstrated that, while later generation (i.e., F_6) were on average less fit than the parents, the novel gene combinations produced resulted in the segregants that could outperform the parental biotypes.

Like *A. barbata*, horseweed (Canada fleabane) [Conyza canadensis (L.) Cronquist; syn. *Erigeron canadensis* L.]is a highly self pollinating, winter annual weed species. It is one of the most widely distributed and problematic weed species throughout much of North America (Weaver 2001) and has evolved resistance to inhibitors of acetolactate synthase (ALS) and enolpyruvyl shikimate 3-phosphate synthase (EPSPS) in multiple states and provinces in the US and Canada(Heap 2023; Smisek 1995; Weaver 2001). In a few regions, in North America and abroad, *E. canadensis* has also evolved resistance to the active ingredient paraquat, a photosystem I electron diverter (Heap 2023). These cases of resistance have often been associated with horticultural systems, such as a orchards and vineyards, where paraquat has been used for the non-selective control of weed species within and between rows of perennial crops (Moretti et al. 2016; Smisek et al. 1998; Yamasue et al. 1992). At present, most of the evidence from studies of paraquat-resistant biotypes suggest that resistance is conferred by a single major gene mechanism that sequesters paraquat away from chloroplasts and into the vacuole (Hawkes 2014). In contrast to the widespread reports of glyphosate resistance in this species (Beres et al. 2020; Heap 2023; Page et al. 2018), paraquat resistance is very much localized to a few regions within North America, specifically the US states of California, Mississippi, Delaware and Oregon, and the Canadian province of Ontario.

In this study, we use paraquat resistant biotypes of *E. canadensis* as a model system for exploring the role of transgressive segregation in the inheritance of herbicide resistance and the biomass characteristics that are often used in the evaluation of resistance. By examining resistant

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biotypes from California and Ontario, we address the question of whether similar resistance mechanisms have evolved in these geographically disparate biotypes. In addition, by assessing the progeny of reciprocal crosses between these two resistant biotypes and a susceptible biotype (also from Ontario), we explore the relative impact of genetic divergence and directional selection on the expression of transgressive segregation. Finally, we evaluate how the expression of transgressive segregation changes when the segregating progeny of these crosses are challenged with the original selective agent, paraquat.

Materials and Methods

Plant material

Three biotypes of *E. canadensis*, two paraquat resistant (R1 and R2) and one susceptible (S), were selected as parents for reciprocal crossing and dose response experiments described herein (Table 1). The paraquat susceptible biotype was previously used to produce a chromosome scale draft genome of *E. canadensis* (Laforest et al. 2020), while the progenitor populations of the resistant biotypes have been characterized in previous studies (Moretti et al. 2016; Smisek et al. 1998).

Dose response

A dose response assay was conducted to confirm the response of the parental biotypes to paraquat (Gramoxone, 200 g a.i. L^{-1}, Syngenta Canada Inc., Guelph, ON). The experiment was established as a completely randomized design with seventeen doses of paraquat (0, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50, 100, 200, 400, 800, 1,600, 3,200, 6,400, 12,800 and 25,600 g a.i. ha^{-1}), four replicates and was repeated in time. The experimental unit consisted of 4 subsample rosettes of *E. canadensis* that were approximately 5cm in diameter. Experimental units were sprayed in an enclosed automatic spray chamber calibrated to deliver 210L ha^{-1} at 276 kPa through a stainless steel even spray nozzle (8002E SS), 40 cm above the plant canopy. Fourteen days after treatment (DAT), the survival of each individual plant within a pot was recorded prior to harvesting the above ground biomass. Plants were considered to be alive if the apical meristem was green in color or if there was evidence of new growth. The four subsamples per pot were harvested and dried together in a forced air dryer at 65°C for 7d prior to being weighed.

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Crossing

The three biotypes of *E. canadensis* (described in Table 1) were used as the parental material in the creation of reciprocal crosses. Seedlings of each biotype were propagated in a greenhouse at the Harrow Research and Development Center (HRDC) with a day/night thermoperiod of 25/20 °C, respectively, and a 16hr photoperiod. Once rosettes reached approximately 5 cm in diameter, seedlings of biotype R1 and R2 were sprayed with 200 g ha^{-1} paraquat in an enclosed spray chamber as previously described. Ten to fourteen days later, seedlings of all three biotypes were placed into cold storage for 4 to 6 weeks at 4°C to meet any vernalization requirements for the transition to reproductive growth (i.e., bolting). Once removed from cold storage, surviving seedlings were transplanted into larger pots and were left to grow to the reproductive stage in greenhouses under the same conditions as described above.

Leaf tissue was collected from each of the prospective parental plants before crossing began. A single sequence repeat (SSR) marker (HW29) (Okada et al. 2015) was used to genotype each individual, with the parental biotypes known to produce PCR products of varying length (i.e., R1 = 148 to 160 bp, R2 = 190 to 195 bp, and S = 170 to 180 bp). DNA was extracted from approximately 20mg of that leaf tissue using a Macherey-Nagel NucleoSpin Plant II kits (Macherey-Nagel Inc., Bethlehem, PA) following the manufacturer's protocol. The PCR reaction cocktail contained the following: 10µl of 2X Taq FroggaMix master mix, 7.6µl nuclease-free H_2O, 0.7µl of 4uM HW29 primer, 0.7µl of 4uM EU47 primer, and 1.0µl (10 ng/µl) DNA for a total reaction volume of 20µl. Amplification was performed with the following cycling profile: denaturation: 94°C for 5 minutes, followed by 35 cycles of 30 seconds at 94°C, annealing: 53°C for 45 seconds and 45 seconds at 72°C, followed up by an extension of 10 minutes at 72°C. PCR products were visualized on a 2.5% agarose gel containing 5% nucleic acid staining solution (RedSafe, FroggaBio Toronto, Canada) along with a 100bp DNA ladder (FroggaBio Toronto, Canada). Only individuals shown to be homozygous with HW29 (i.e., those with only a single band) and with the proper product size for a given parental biotype were used in subsequent crosses.

The crossing methods used in the current study closely follow those outlined by Zelaya et al. (2004). In brief, each cross was initiated by selecting pairs of individuals from the desired set of parental biotypes whose floral initiation was in close synchrony. As noted by Zelaya et al.

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(2004) and Weaver (2001), *E. canadensis* capitula (inflorescence) contain pistillate ray and perfect disk florets (flowers) (Figure 1). Controlled crossing between individuals is thus achieved by the removal of the disk florets (i.e., emasculation, Figure 1D) using forceps and the transfer of the desired pollen to the ray florets. To assess the efficiency of our emasculations, a capitulum was selected at the top of each plant to serve as a negative control (i.e., was emasculated and covered to prevent outcrossing). Capitula used for crossing were selected at random and emasculated when they reached the appropriate stage of development (Figure 1A). The remaining ray florets were cross pollinated once a day, every day for seven to ten days until the capitula closed, indicating the onset of seed maturation. Reciprocal pollen transfer was achieved by emasculating donor capitula from an individual of the desired parental biotype and brushing these mature perfect disk florets (Figure 1B and C) on the ray florets of the emasculated recipient capitulum.

The number of F_1 achenes (hereafter referred to as seeds) produced by each cross ranged from one to 20. When mature, F_1 seeds were harvested they were immediately set to germinate in an incubator. Seeds were placed into a Petri dish lined with moist blue blotter paper (steel-blue germination blotters, Anchor Paper, St. Paul, MN) and incubated under the following conditions: a 25/10°C day/night thermoperiod, 60% relative humidity and a 14-hour photoperiod. Once germinated, seedlings were transplanted and grown in a greenhouse under the same conditions as previously described. All seedlings were genotyped with the HW29 SSR marker (as describe above) and only those demonstrated to be heterozygous (i.e., 2 bands), with bands corresponding to the appropriate parental biotypes, were retained. All heterozygous F_1 individuals were once again cold acclimated to accelerate bolting. The progeny from separate crosses were segregated by greenhouse compartment and all individuals were covered with DelNet pollination bags (DelStar Technologies inc, Austin, TX) prior to flowering to ensure self pollination and facilitate the collection of the F_2 seed. Seeds from each plant were kept as separate F_2 families.

Inheritance of paraquat resistance

The F_2 progeny arising from the reciprocal crosses among the three parental biotypes were screened at discriminating doses (i.e., the lowest dose that provides 100% mortality of the most susceptible parent in a specific cross based on dose response survival curves; see section above). These doses were 400 g ha^{-1} for the S x R2 cross and 12, 800 g ha^{-1} and for the R1 x R2 cross.

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Seedlings of the F_2 generation and their parental biotypes were propagated in greenhouse plug flats under the conditions previously described. Once the rosettes reached 5cm in diameter, experimental units were created by transplanting rosettes as plugs in a new flat that contained an individual plant of each parent for a given cross and an F_2 individual produced from each of the reciprocal crosses between these parental biotypes (i.e., 6 or 8 F_2 rosettes + 2 parental rosettes per tray). For example, for the R2 x S cross there were six F_2 families created and 33 replicates were screened at the discriminating dose, resulting in a total of 198 F_2 individuals. At 21 DAT, the survival of the rosettes was recorded. The phenotype of surviving F_2 individuals closely resembled that of one or the other parental resistant biotypes; no intermediate phenotypes were observed. The aboveground biomass of all individuals surviving at discriminating doses was harvested and samples were dried in a forced air dryer at 65°C for 7d prior to biomass being recorded.

Seed viability

While crosses between the R1 and S parental biotypes successfully produced an F_1 generation, the self pollination of these F_1 consistently failed to produce germinable F_2 progeny. When examined under a microscope with 100x magnification, a noticeable difference in seed integrity was observed between known viable seed and the seeds from these crosses (H. Hickmott, personal observation). A tetrazolium chloride assay was subsequently used to examine the viability of parental and F_2 seed (Peters and Lanham 2000). Fifty seeds of each F_2 family and parental biotype were counted and placed into individual Petri plates; there were four replicates of each. A 10 mL volume of a 1% W/V solution of tetrazolium chloride was added and plates were placed in a growth cabinet at 30°C in complete darkness. After 24 hours, the Petri plates were removed from the growth cabinet and seed viability was rated under a dissecting scope.

Biomass accumulation under unsprayed conditions

Seedlings of the three parental biotypes and the two successful F_2 generations were propagated under greenhouse conditions as described above. Fifty individuals of each F_2 family and one hundred individuals of each parental biotype were propagated to a size like that utilized in the dose response and inheritance studies described above (i.e., to approximately 5cm in

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diameter). Four weeks after emergence, the above ground biomass was harvested, dried in a forced air dryer at 65°C for 7d prior to biomass being recorded.

Statistical analyses

The parental dose response was conducted as completely randomized design with four replications, and two repetitions in time. Survival and aboveground biomass data were used for dose response analyses using PROC NLIN in SAS Version 9.4 (SAS Institute, Cary, NC). Data were fit to a log-logistic model (eq. 1) (Seefeldt et al. 1995), where D is the upper response limit bounded at ≤ 100; C is the lower response limit, LD_{50} and GR_{50} are the herbicide dose that results in 50% reduction in survival and aboveground biomass, respectively; and *b* is the slope at the inflexion point.

$$f(x) = C + \frac{D-C}{1+exp\,[b(log\,(x) - log\,(LD_{50}))]} \quad (1)$$

At each discriminating dose, segregation ratios in the F_2 generation were analysed by χ^2 test (Hayes and Immer 1942). The χ^2 test for homogeneity was performed to determine whether segregation data could be combined across families. Biomass accumulation of parental and F_2 families sprayed with a discriminating dose or from unsprayed conditions were analyzed with analyses of variance. For the unsprayed data set, a one-way analysis of variance (ANOVA) was conducted in PROC MIXED with biotype or family as a fixed effect and replicate as a random effect. For the R1xR2 cross, the biomass accumulation at the discriminating dose was analyzed in a similar manner. In the SxR2 cross, however, the S parent was completely controlled at the discriminating dose. Thus, only the biomass from the R2 parent and the F_2 generation were included in the analysis. Finally, to assess the impact of inheritance of the resistance trait on the expression of transgressive segregation, the ANOVA for biomass accumulation at each discriminating dose were repeated using data sets where only survivors were included.

Results and Discussion

Response of parental biotypes to paraquat

The phenotypic response of the two resistant biotypes (R1 and R2) to paraquat was notably different. After treatment with paraquat, the older leaf tissue of R2 individuals became necrotic within days while the young leaves and the apical meristem remained green and

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continued to produce new tissue (Figure 2). Individuals of the R1 biotype, however, displayed no visual herbicide symptomology. The level of resistance also varied among biotypes, as evidenced by their respective LD_{50} (Figure 3). The dose of paraquat required to provide 50% control ranged from 10,749 g ha^{-1} for R1 to 3,511 and 73 g ha^{-1} for R2 and S, respectively. Based on these results, the R1 biotype exhibited a resistance factor of 148-fold while the R2 biotype exhibited a resistance factor of 48-fold, relative to our S control. The response of aboveground biomass was similar to that measured for survival across the studied biotypes (Figure 4). The GR_{50} of the three biotypes ranged from 832 g ha^{-1} for R1 to 56 and 13 g ha^{-1} for R2 and S, respectively.

Inheritance of paraquat resistance

In this study, we examined the inheritance of paraquat resistance in segregating F_2 generations created from the reciprocal crosses of two known resistant biotypes and a paraquat susceptible biotype (Table 1). The self-fertilization of F_1 plants from these three reciprocal crosses all produced F_2 progeny, however, those of the S x R1 cross were uniformly non-viable. This cross was repeated twice in time, producing a total of 21 F_2 families, all of which were non-viable. Results of a tetrazolium chloride assay indicated that 3 of the F_2 families from the cross of S x R1 had ≤1% viable seed, whereas the other 18 had no viable seed (Figure 5). In contrast, all parental biotypes germinated consistently, and results of the tetrazolium assay indicated that their seed lots contained 64 to 68% viable seeds (data not presented).

Segregating F_2 generations were successfully created from the reciprocal crosses between S and R2 and R1 and R2 and these were examined at appropriate discriminating doses based on the dose responses of their respective parental biotypes (Table 1, Figure 3). At a paraquat dose of 400 g ha^{-1}, the survival of F_2 individuals arising from the cross of S x R2 approached 67% (133/198) (Table 2). The χ^2 test of the pooled F_2 indicated that these results deviated from the 3:1 ratio expected under the assumption of monogenic inheritance of the paraquat resistance trait. Similarly, at a paraquat dose of 12,800 g ha^{-1}, survival in the F_2 progeny of the R1 x R2 cross approached 61% (118/192) and the χ^2 test of the pooled F_2 also indicated that these results deviated from the expected 3:1 ratio (Table 3). These results differ from previous studies of paraquat resistance in *E. canadensis* where segregation ratios of 3:1 (R:S) have been reported (Smisek 1995; Yamasue et al. 1992). When tested against several digenic ratios, results from S x R2 cross fit an 11:5 ratio while the results from the R1 x R2 did not fit any of the tested ratios

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(Table 4). A digenic ratio of 11:5 was similarly observed by Okada and Jasieniuk (2014) in their study of glyphosate resistance in a Californian biotype of hairy fleabane [*Conyza bonariensis* (L.) Cronquist], a closely related species to *E. canadensis*. In this two locus model, resistance alleles work additively across loci and at least two doses of the resistance allele are required to produce the resistant phenotype.

Herbicide resistance and transgressive segregation

When the F_2 generations created in this study were characterized with respect to their biomass accumulation it was clear that the mean and range of individual sizes in the F_2 generation exceeded that observed in either parental biotype (Figure 6). In the S x R2 cross for example, not only did the mean biomass accumulation in the F_2 exceed that of the parental biotypes by 51 and 63%, respectively, but the range of observed values was nearly double that for either parent. Results for the R1 x R2 cross were nearly identical, with the mean aboveground biomass of the F_2 exceeding that of either parent by 50 and 52%, respectively, which indicated a doubling of the range for the trait as compared to the parents.

When the segregating F_2 generations and their parental biotypes were sprayed with discriminating doses of paraquat there were notable differences in the means and ranges of biomass accumulation after application (Figure 7 & 8). In the S x R2 cross, the selected dose was perfectly discriminatory and there was no difference in the mean aboveground biomass of the F_2 and the resistant parent of the cross (Figure 7A). There was, however, a notable difference in the range of above ground biomass values in the F_2 generation, which exceeded the observed range in the R2 parent by 58%. This result was comparable the transgressive segregation in biomass accumulation observed under unsprayed conditions (Figure 6). When only the surviving individuals are considered (i.e., those with the resistance trait), the mean aboveground biomass of the F_2 generation was 52% greater than that of the R2 parent at 21 DAA (i.e., 97 vs 64 g plant^{-1}) and the range of values exhibited by the F_2 was 61% greater than that observed in R2, further emphasizing the expression of transgressive segregation in the resistant members of the F_2 generation (Figure 7B).

For the R1 x R2 cross, the selected dose was not completely discriminatory, and this reflected a difficulty in selecting an appropriate dose given that both parental biotypes exhibited

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some degree of resistance to paraquat (Figure 3). The biomass accumulation 21 DAA varied among the F_2 and the parental biotypes, such that individuals of the more resistant parent (i.e., R1) were on average 2.5 and 12 times larger than the F_2 and the less resistant parent (i.e., R2), respectively. In contrast to the results from the S x R2 cross (discussed above), the mean and range of biomass accumulation in the F_2 generation was intermediate to the two parental resistant biotypes and there was no evidence for transgressive segregation (Figure 8A). These conclusions did not change when only the surviving individuals were considered (Figure 8B).

Results of the current study highlight three potential outcomes arising from crosses amongst resistant and susceptible biotypes of *E. canadensis*: (i) hybrid sterility, (ii) transgressive segregation and (iii) inheritance intermediate to parents exhibiting contrasting phenotypes. At present, it is unclear why the hybrid of a susceptible biotype from Ontario and a resistant biotype form California repeatedly failed to produce viable seed. It is possible that gametophytic incompatibility between the genomes of the S and R1 parental biotypes during F_1 self-pollination resulted in pollen inviability or in the incomplete development of the zygote (McClure and Franklin-Tong 2006; Newbigin et al. 1993; Ouyang et al. 2010). Interestingly, hybrid sterility was not observed in the hybrid of the two resistant biotypes, also originating from California and Ontario. The latter suggests that the observation of hybrid sterility was specific to the combination of parental linages used in the cross and did not reflect broader geographically based incompatibility (Baack et al. 2015). Further research is required to explore the frequency of this phenomenon in *E. canadensis* in order to understand its role in shaping intraspecific population dynamics.

Erigeron canadensis is considered to be a highly self-fertilizing species with outcrossing rates ranging between 2 and 13% (Smisek 1995). We would therefore anticipate transgressive segregation to be expressed more frequently in *E. canadensis* than, for example, in a highly outcrossing species such as common ragweed (*Ambrosia artemisiifolia* L.) (Friedman and Barrett 2008; Rieseberg et al. 1999). Our results clearly support this prediction, with all successful crosses exhibiting transgressive segregation in biomass accumulation. In their review of transgressive segregation, adaptation and speciation, Rieseberg et al. (1999) also predicted that transgressive segregation would be: (i) positively correlated with genetic divergence of the parental lineages and (ii) is less likely to be observed in lineages with a shared history of

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directional selection. Results of our study mostly agree with these two predictions, however, our results show conclusions may not be as straightforward depending on the parents' histories.

Based on geographic distance alone, we anticipate a greater potential for transgressive segregation in the progeny of R1 and R2 than from S and R2 (Table 1). The geographic distance between R1 and R2 approaches 3,500 km and spans a continental divide, whereas S and R2 are only separated by approximately 300 km (Table 1). For most species, this later distance would still represent a significant barrier to gene flow between the regions, yet propagules of *E. canadensis* have been observed to disperse up to 500 km in a single dispersal event when travelling in the planetary boundary layer (Shields et al. 2006). While this propensity for long distance dispersal raises the potential for gene flow between S and R2, it is also counterbalanced by the low frequency for outcrossing for plants in close proximity (Smisek et al. 1998) and the observation that the vast majority (i.e., >99%) of propagules disperse within a 100m of the parent plant (Dauer et al. 2007). The observation of transgressive segregation in segregating generations of both crosses (in the absence of paraquat) suggests that there was genetic diversity among the parental biotypes studied and this resulted in novel allelic combinations in the progeny.

The geographic distance between biotypes R1 and R2 belies the fact that they share a history of selection with the herbicide paraquat. Both biotypes originated in orchard production systems, in California and Ontario, respectively, where paraquat was applied for weed control multiple time per growing season. It is clear from our dose response data that, while both biotypes are resistant to paraquat, the Californian biotype (R1) is approximately three times more resistant than the Ontarian biotype (R2). Our results also suggested that the traits conferring resistance in these biotypes were both polygenic, although it remains unclear whether they result from the same molecular mechanism(s). Given this shared history of selection with paraquat it is plausible to hypothesize that a similar resistance mechanism may have been selected in these biotypes, resulting in a reduction of diversity in the genomic loci where genes involved in resistance are found as well as other genes that are in linkage disequilibrium. This fixation of alleles is predicted to decrease the expression of transgressive segregation in the progeny of lineages that share a history of directional selection (Rieseberg et al. 1999). The current study provided a test of such hypothesis, both in the absence and presence of the original selective agent (i.e., paraquat).

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In the absence of paraquat, our results clearly refute the prediction that transgressive segregation would be reduced in the progeny of biotypes with a shared history of directional selection. Transgressive segregation was not only evident, but was of similar magnitude in the segregating progeny of the S x R2 and R1 x R2 crosses. However, when paraquat was applied to these same segregating generations, transgressive segregation was absent from the progeny of R1 x R2. While these results (with and without the original selective agent) are seemingly contradictory, we contend that they are in fact supportive of the original prediction of Rieseberg et al. (1999) and are underpinned by the diversity of complementary alleles among biotypes, the number of loci involved, the gene expression under selective agent and without it, the intensity of selection pressure and fixation of alleles following selection. When the progeny of the R1 x R2 cross were challenged with paraquat, it was at a discriminating dose that was selected to eliminate the R2 biotype. As a result, survivors would have to possess the complete resistance from R1 and/or alleles from R2 that were common to the R1 resistance mechanism. It is also important to note that the resistance in R2 provided less protection than that in R1, therefore, may not have reached fixation at all the loci contributing to paraquat resistance in this species. Therefore, we hypothesize that the expression of transgressive segregation in the R1 x R2 progeny in the absence of paraquat stems from the fact that there remains allelic differences between the parental biotypes at the loci conferring paraquat resistance. It is only when paraquat is applied that we can truly see the effects of selection on the fixation of alleles and the subsequent impact on transgressive segregation.

In summary, transgressive segregation in life history traits contributing to survival and reproduction can play an important role in determining fitness. Segregants that exceed the parental values in such critical traits may represent beneficial new gene combinations that are fitter than their respective resistant parental genotypes. The relative fitness of a particular genotype, however, depends on the environment to which it is exposed (Leon et al. 2021), such that transgressive segregants in one environment may not necessarily exceed the parental genotypes in another (Johansen-Morris and Latta 2006). Hybridization among diverse biotypes of *E. canadensis* clearly produces transgressive segregants; a result that supports the prediction that transgressive segregation is frequently expressed in self-fertilizing lineages and is positively correlated with the genetic diversity of the parental genotypes (Rieseberg et al. 1999). When these hybrid progenies were exposed to a new environment, one in which past agents of

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directional selection were not present, segregants were observed in the F_2 generation regardless of parental identity or history. In this environment, which would be akin to a year where herbicide rotation was practiced, we would predict that many of the recombinant genotypes would be fitter than either of the parental biotypes. Conversely, if these hybrid progenies were returned to the parental environment with exposure to paraquat, the identity of fittest genotype (i.e., parent or segregant) would depend on the history of directional selection in the parental lineages and the dose to which the hybrid progeny was exposed. When both parental lineages share the same history of directional selection it does reduce the likelihood of transgressive segregation, however, its expression in this case depends on the strength of selection exerted on the progeny.

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Competing interests

The author(s) declare none.

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Table 1. Origins of *E. canadensis* biotypes

Biotype	Collection location	Lat/Lon	Cropping system	Previous characterization	Citation
S	Welland, ON, Canada	43.00421°N, 79.36771°W	Field crops	Paraquat susceptible	Page et al. 2018; Laforest et al. 2020
R1	Discovery Bay, CA, USA	37.9085 °N, 121.6002° W	Almond orchard	Paraquat resistant	Moretti et al 2016; B. Hanson personal comm.
R2	Harrow, ON, Canada	42.033847°N, 82.894238°W	Peach orchard	Paraquat resistant	Smisek et al. 1998, Weaver et al. 2004

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Table 2. Segregation of resistance in F_2 families from crosses between *E. canadensis* biotypes S and R2 twenty-one days after treatment with paraquat at 400 g ha^{-1}.

Biotype	Segregation by phenotype		χ^2 (3:1)	df	*P*-value
	Resistant	Susceptible			
S	0	33			
R2	29	4			
S x R2 – A[a]	24	9	0.09	1	0.763
R2 x S – A	21	12	2.27	1	0.132
S x R2 – B	21	12	2.27	1	0.132
R2 x S – B	25	8	0.01	1	0.920
S x R2 – C	23	10	0.49	1	0.482
R2 x S - C	19	14	5.34	1	0.021
Total of 6 F_2 families	133	65	6.47	1	0.011
Test of heterogeneity among F_2 families			4.01	5	0.547

[a]Within each cross, the first parental biotype listed was the pollen donor and the second, the pollen recipient. Families sharing a letter represent crosses between the same 2 parental individuals.

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Table 3. Segregation of resistance in F_2 families from crosses between *E. canadensis* biotypes R1 and R2 twenty-one days after treatment with paraquat at 12,800 g ha^{-1}.

Biotype	Segregation by phenotype		χ^2 (3:1)	df	*P*-value
	Resistant	Susceptible			
R1	61	13			
R2	14	60			
R1 x R2 – A[a]	15	9	2.00	1	0.157
R2 x R1 – A	18	6	0.00	1	1.000
R1 x R2 – B	12	12	8.00	1	0.005
R2 x R1 – B	13	11	5.56	1	0.018
R1 x R2 – C	18	6	0.00	1	1.000
R2 x R1 – C	13	11	5.56	1	0.018
R1 x R2 – D	17	7	0.22	1	0.637
R2 x R1 - D	12	12	8.00	1	0.005
Total of 8 F_2 families	118	74	18.78	1	<0.001
Test of heterogeneity among F_2 families			10.56	7	0.159

[a]Within each cross, the first parental biotype listed was the pollen donor and the second, the pollen recipient. Families sharing a letter represent crosses between the same 2 parental individuals.

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Table 4. Segregation of paraquat resistance in F_2 populations and expected ratios under four two-locus models and the *P* values from chi-square tests for goodness of fit.

Cross	Dose (g ha^{-1})	Resistant	Susceptible	Digenic ratios			
				15:1	11:5	7:9	13:3
				P-value			
S x R2	400	133	65	$7.5E^{-54}$	0.63	$3.9E^{-07}$	$3.9E^{-07}$
R1 x R2	12,800	118	74	$2.7E^{-76}$	0.03	$7.5E^{-07}$	$2.1E^{-12}$

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Figure 1. Stages of capitulum development in *E. canadensis*. Panel A presents the capitulum containing both disk and ray florets at the appropriate stage for emasculation. Panel B and C represent intact capitulum with mature disk florets for use as pollen donors. Panel D presents the capitulum post-emasculation (i.e., disk florets removed and ray florets remaining).

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Figure 2. Parental resistant biotype (R2) of *E. canadensis* treated with 1,600 g ha $^{-1}$ paraquat, pictured 24 hours after treatment (A) and 14 days after treatment (B).

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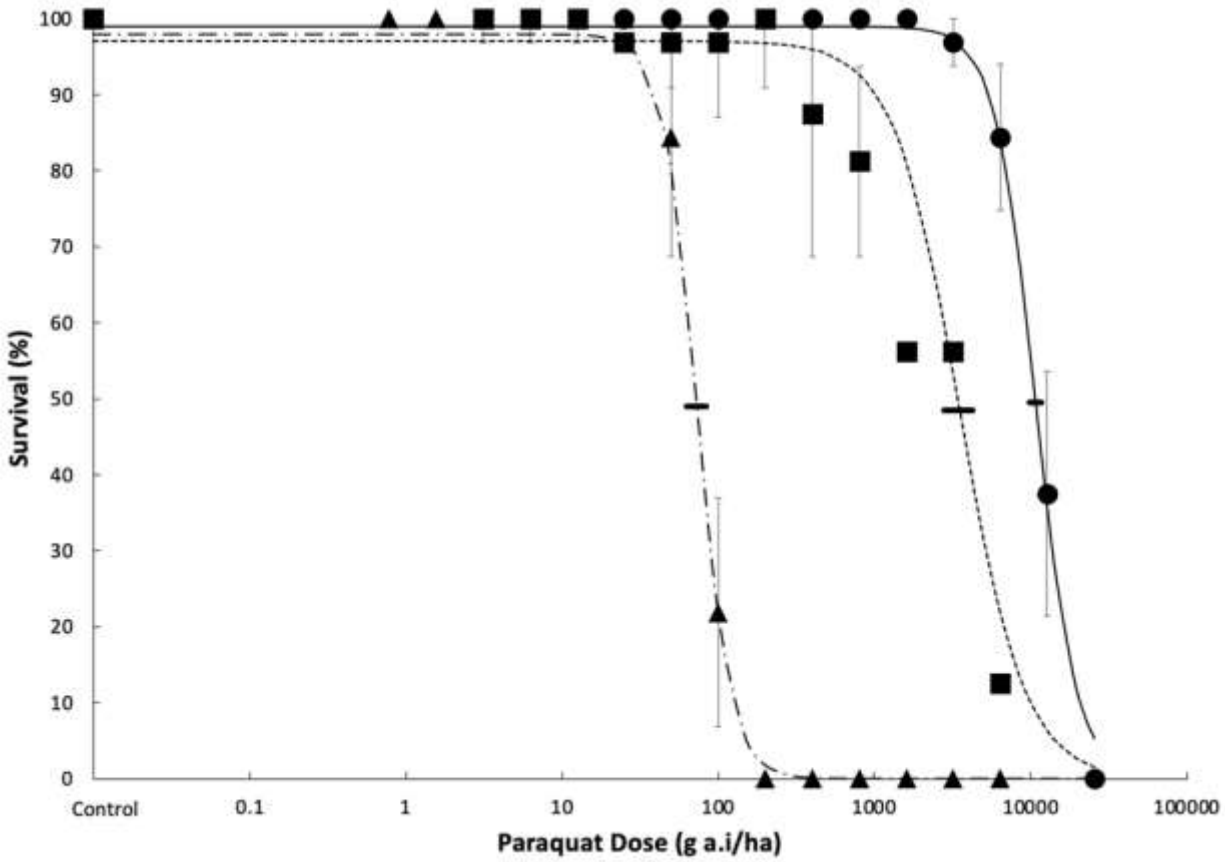

Figure 3. Survival of three biotypes of *E. canadensis* (S (▲, – ● – ● –), R2 (▪, ----), R1 (● , ——)) as influenced by paraquat dose. Data points represent the mean survivorship of 4 plants per experimental unit at 14 days after treatment. Horizontal error bars represent the 95% confidence interval at LD_{50}. Vertical error bars represent the standard error of the mean. A four-parameter log-logistic equation ($f(x) = C + D - C/1 + \exp[b(\log x) - \log(LD_{50})]$) was fit to R1 (C = 0, D = 99, LD_{50} = 10,749, b = 3.3), R2 (C = 0, D = 197, LD_{50} = 3,511, b = 2.06), and S (C = 0, D = 98, LD_{50} = 73 b = 3.97).

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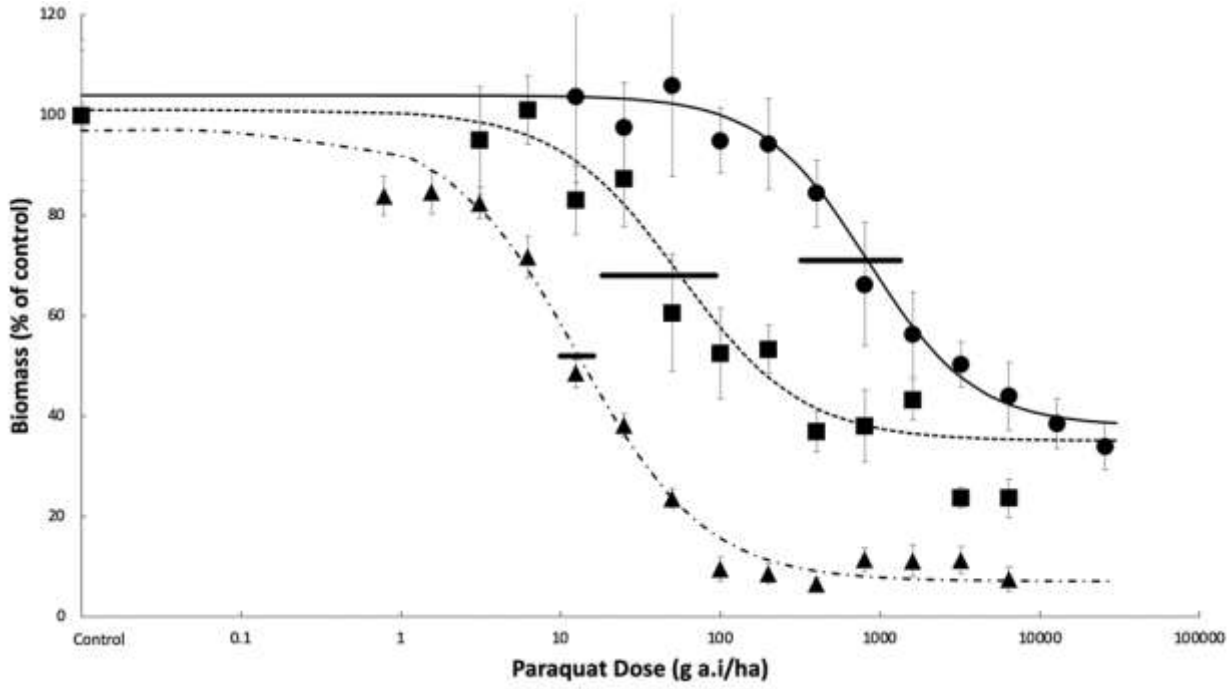

Figure 4. Dose response of three biotypes of *E. canadensis* (S (▲, – ● – ● –), R2 (□, ----), R1 (● , ——)) as influenced by paraquat dose. Data points represent the mean biomass of 4 plants per experimental unit at 14 days after treatment. Horizontal error bars represent the 95% confidence interval at GR_{50}. Vertical error bars represent the standard error of the mean. A four-parameter log-logistic equation Dose response curves were generated via non-linear regression analysis. A four-parameter log-logistic equation ($f(x) = C + D - C/1 + \exp[b(\log x) - \log(GR_{50})]$) was fit to R1 (C = 38, D = 104, GR_{50} = 832, b = 1.3), R2 (C = 35, D = 101, GR_{50} = 55.8, b = 1.13), and S (C = 7, D = 97, GR_{50} = 12.9, b = 1.1).

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Figure 5. Achenes of *E. canadensis* 24h after treatments with a 1% tetrazolium chloride solution. Top row, left to right are as follows: R1, S, R2. Second row are representative achenes from 3 F_2 families arising from the cross of S x R1. Achenes of *E. canadensis* are on average 1-2mm long (Weaver 2001).

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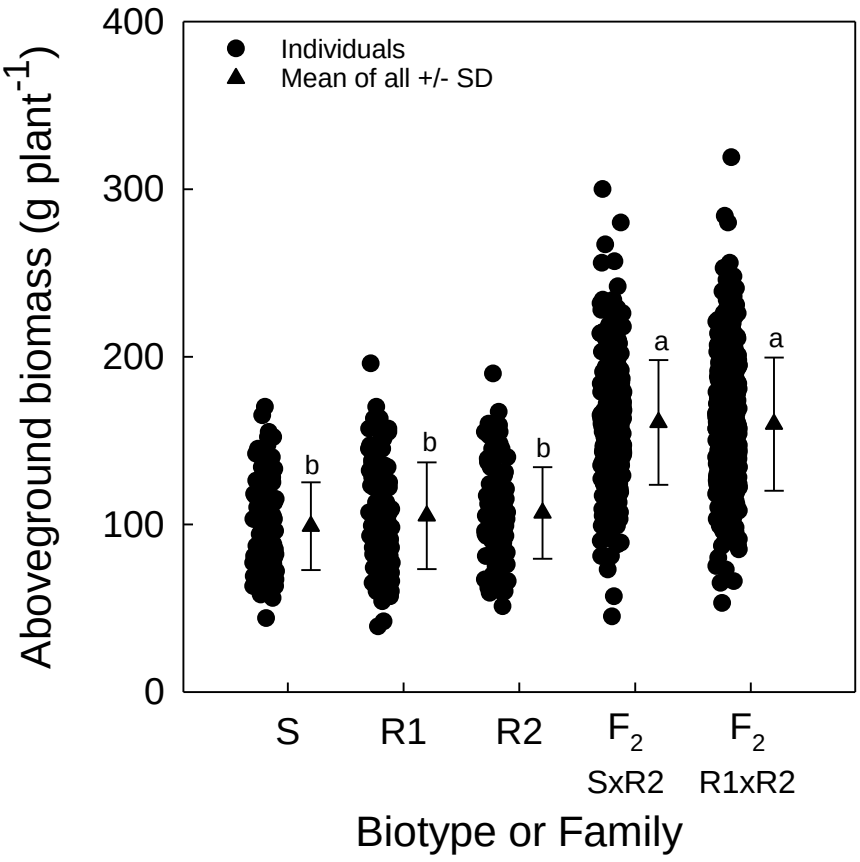

Figure 6. Aboveground biomass of *E. canadensis* parental biotypes (S, R1 and R2) and the F_2 progeny of their successful crosses in the absence of paraquat. Mean values bearing the same letters are not significantly different at $p < 0.05$ according to Tukey's HSD.

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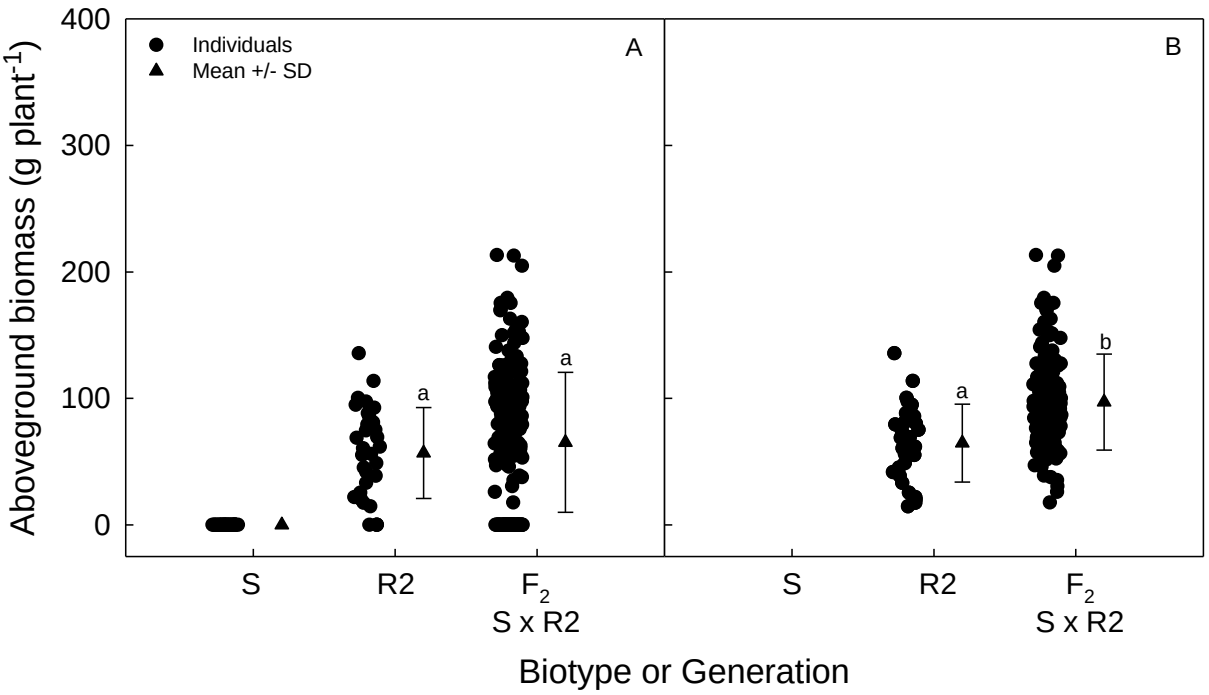

Figure 7. Aboveground biomass of *E. canadensis* parental biotypes S and R2, and their F_2 progeny twenty-one days after application of paraquat at 400 g ha^{-1}. Panel A contains all individuals, whereas Panel B contains only survivors. Mean values within a panel bearing the same letters are not significantly different at $p < 0.05$ according to Tukey's HSD.

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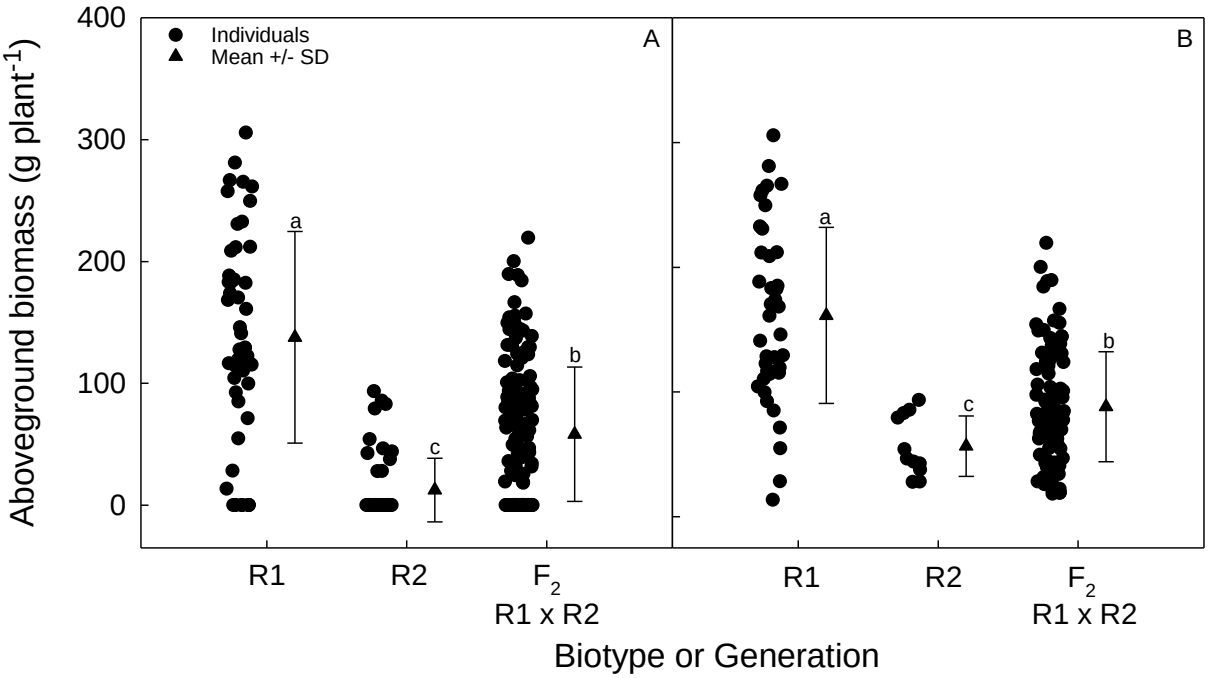

Figure 8. Aboveground biomass of *E. canadensis* parental biotypes R1 and R2, and their F_2 progeny twenty-one days after application of paraquat at 400 g ha^{-1}. Panel A contains all individuals, whereas Panel B contains only survivors. Mean values within a panel bearing the same letters are not significantly different at $p < 0.05$ according to Tukey's HSD.

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