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EVALUATING THE INTERACTIVE ROLES OF SOIL NUTRIENTS AND POLYPLOIDY ON COMPETITIVE OUTCOMES OF CHAMERION ANGUSTIFOLIUM

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EVALUATING THE INTERACTIVE ROLES OF SOIL NUTRIENTS AND
POLYPLOIDY ON COMPETITIVE OUTCOMES OF *CHAMERION*
ANGUSTIFOLIUM

By

Angela M. Walczyk

A THESIS

Submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

In Biological Sciences

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This thesis has been approved in partial fulfillment of the requirements for the Degree of
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Preface

The contents of this thesis were completed with collaboration from Dr. Erika Hersch-Green of Michigan Technological University. Writing, data collection, and data analysis was completed by me. Dr. Hersch-Green assisted with experimental design, data analysis, and chapter edits. Chapter 1 of this thesis is in preparation to be submitted to the Journal of Ecology, and Chapter 2 to be submitted to the Journal of Evolution.

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Abstract

Newly formed polyploids face strong barriers preventing their establishment, but despite these barriers polyploidy is prevalent among angiosperms being a major mechanism of adaptation and speciation. Morphological and phenological differences between diploids and related polyploids often results in different ecological tolerances among cytotypes. Differences in competitive abilities brought on by genome duplication can vary with abiotic and biotic environmental conditions and influence polyploid establishment. In my theses, I test the overall hypothesis that soil nutrient availability and polyploidy interact to affect competition and performance of Fireweed plants (*Chamerion angustifolium*), which differ in ploidy levels. Additionally, I examined whether insect feeding damage is influenced by soil nutrient availability and polyploidy.

In the first chapter, I detail a greenhouse experiment, in which I tested how soil nitrogen and ploidy level interact to affect plant competitive outcomes and performance of diploid and autotetraploid plants (cytotypes). To do this, in a greenhouse experiment, I grew cytotypes alone or with another plant of either the same or a different cytotype under low and high soil nitrogen conditions. To examine whether herbivory may affect competitive outcomes, and if soil nitrogen supply and/or ploidy level influenced insect feeding damage, we conducted a leaf-choice insect bioassay and a whole-plant insect bioassay. I found that a competitor's ploidy level influenced plant growth traits as plants grown with tetraploids were generally smaller, but soil nitrogen availability did not differentially affect this competitive outcome. Additionally, insect damage was not influenced by competition nor soil nitrogen supplies.

The second chapter details a greenhouse experiment where I examined how nitrogen and phosphorus availability and ploidy level interact to affect plant competitive outcomes and the performance of diploid, established tetraploid, and neotetraploid cytotypes. I grew the plants alone or in competition with the same or a different cytotype under low and high soil nutrient concentrations. I also conducted a leaf-choice insect bioassay and a whole-plant insect bioassay to test if competitive outcomes were affected by herbivory, and if a plant's ploidy level and soil nutrient supplies influenced insect feeding damage. We found that whole genome duplication effects competitive outcomes and plant-herbivore interactions, but these outcomes vary depending on nutrient supply.

Overall, our findings suggest that polyploids possess some traits related to competitive ability, and that these traits may have been selected for in natural populations. We conclude that soil nutrient availability plays a role in mediating the competitive outcomes between cytotypes and could be an important factor facilitating polyploid population establishment.

Chapter 1: Investigating the competitive outcomes of diploid and autotetraploid *Chamerion angustifolium* cytotypes under different nitrogen supplies

1.1 Abstract

The ecological and evolutionary mechanisms leading to the establishment and structuring of polyploid populations are not well known. Morphological and physiological differences between cytotypes may affect competitive and consumptive forces by influencing how plants interact with their biotic and abiotic environment. Due to their larger genome size, polyploids are expected to require more nitrogen than diploids; therefore, polyploids are expected to perform poorly and lack competitive vigor in nitrogen scarce conditions, potentially giving diploids a competitive advantage. In a greenhouse study, we tested the hypothesis that a cytotype's performance traits and foliar damage from feeding insects are differentially influenced by the ploidy level of its competitor under low and high nitrogen supplies using diploid and autotetraploid *Chamerion angustifolium* (fireweed). Overall, our hypothesis was partially supported as we found evidence that some growth and reproductive traits are suppressed when plants are grown with a tetraploid under both low and high nitrogen treatments. Tetraploids were significantly larger than diploids in low nitrogen treatments, and diploids exhibited the most clonal growth in high nitrogen treatments. Our results suggest that tetraploids may possess performance traits related to competitive ability regardless of nitrogen

supplies, and that nitrogen scarcity does not necessarily have a negative effect on tetraploid performance, which may imply that established tetraploid plants experienced strong selective forces resulting in these competitive traits.

1.2 Introduction

Polyploidy, or whole genome duplication, is one of the most important mechanisms in terrestrial plant evolution and diversification (Soltis and Soltis 1999, Husband 2000, Soltis and Soltis 2009), contributing to the formation of new species and to genetic and phenotypic diversity within species (Masterson 1994, Otto and Whitton 2000, te Beest et al. 2012). Polyploidization also influences ecological tolerances (Soltis and Soltis 1999, Thompson et al. 2014, Thompson et al. 2015) and the ways in which plants interact with their biotic and abiotic environments (Segraves 2017). Polyploids have more DNA per cell compared to diploids, and due to the fact that nucleic acid production requires high quantities of soil nutrients, it is hypothesized that polyploids have a greater nutrient requirement than diploids (Lewis 1985, Leitch and Bennett 2004, Cavalier-Smith 2005, Guignard et al. 2017). Soil nutrient scarcity can have a negative effect on polyploid productivity and growth, potentially affecting the way that diploids and polyploids compete with each other and define ecological niches (Terry et al. 1985, Berdalet et al. 1994). This has been supported by field studies showing that polyploid plants tend to display traits related to a greater competitive ability in nutrient rich soils (Smarda et al. 2013, Guignard et al. 2016). Additionally, spatial separation of diploid and related polyploid populations has been documented in a variety of species (Mosquin and Small 1971, Baack 2004, Schlaepfer et al. 2008, Laport et al. 2012, Peirson et al. 2012, Laport et al. 2013), and it is thought that these distribution patterns result from the influence of ecological tolerances on the competitive outcomes between diploids and polyploids (Collins et al. 2011, Thompson et al. 2014, Thompson et al. 2015). The goal

of this study was to determine if differing soil nitrogen supplies affected the performance traits of diploids and polyploids grown in competition with each other as a way to understand how abiotic factors influence the establishment and structuring of polyploid populations.

Competition among plants (Grime 1977, Grime and Hodgson 1987, Goldberg and Barton 1992, Fraser and Keddy 2005) and plant consumption by herbivores (Crawley 1989, Maron and Crone 2006, Eskelinen et al. 2012, Kempel et al. 2015) are two of the main forces shaping plant community structure. Competition is a complex selective force that plays a key role in defining niches through a plant's ability to suppress a neighboring plant's access to limited resources (Aarssen 1983, Goldberg and Landa 1991, Goldberg 1996, Craine and Dybzinski 2013) or avoid being suppressed itself (Goldberg and Fleetwood 1987, Goldberg and Landa 1991). Herbivory generally has a negative effect on plant primary production, fitness, and competitive abilities, all of which influence plant community structure (Crawley 1989, Wise and Abrahamson 2005, Eskelinen et al. 2012, Kempel et al. 2015). For example, insects feeding on plant foliage can reduce a plant's vigor by reducing its photosynthetic activity and growth rate, while also increasing its susceptibility to pathogenic infection, such a plant would be at a competitive disadvantage when competing with an undamaged neighbor (Crawley 1989). Because the variety and effectiveness of competitive traits is associated with genetic diversity (Aarssen 1983, Aschehoug et al. 2016), and because herbivores are thought to be sensitive to the morphological and physiological differences associated with polyploidy (Nuismer and Thompson 2001, Thompson et al. 2004, Halverson et al. 2008,

Hull-Sanders et al. 2009, Münzbergová et al. 2015), competitive and consumption forces could be especially influential in shaping the population structures of species exhibiting polyploidy. Oftentimes the strength and outcomes of these community-shaping forces are influenced by environmental factors (Dudt and Shure 1994, Aerts 1999, Bale et al. 2002). Because some diploids and polyploids have differing ecological tolerances, abiotic environmental factors may be an important component to the mechanisms shaping polyploid population structure.

Here we evaluate the hypothesis that soil nitrogen supplies differentially effect competitive outcomes and insect consumption patterns between diploid and autotetraploid *Chamerion angustifolium* (L.) Holub (Onagraceae), fireweed. Nitrogen is a globally limited and important abiotic environmental factor in plant physiology due to its role in DNA synthesis, photosynthesis, cellular metabolism, and protein production (Terry et al. 1985, Berdalet et al. 1994, Leghari et al. 2016). Because diploids and tetraploids differ in their nutrient requirements (Lewis 1985, Bennett 2004, Cavalier-Smith 2005, Guignard et al. 2017) and because nitrogen is crucial in nucleic acid formation, it is possible that competitive outcomes between cytotypes are influenced by soil nitrogen availability (Brooker et al. 2005, Chamberlain et al. 2014, Aschehoug et al. 2016), and that polyploid plants experience tradeoffs when investing in nucleic acid formation rather than plant growth. A plant's resource allocation strategy (the proportion of resources allotted to the growth of root, shoot, and/or reproductive tissues) and overall performance under different nutrient conditions can contribute to competitive ability (Tilman 1986, Tilman and Cowan 1989, Aerts 1999). For example, plants adaptive to

high nutrient environments can express greater nutrient uptake kinetics through root plasticity (Jackson et al. 1990), which may result in the competitive advantage of a faster growing plant that is able to deplete soil nutrients before its slow-growing neighbor can access them (Aerts 1999). Plants that are adaptive to nutrient poor environments often develop characteristics to reduce nutrient loss (i.e. long tissue life; slow growth (Eckstein and Karlsson 1997)), which can be advantageous against plants lacking these characteristics. Plant-insect interactions can also be influenced by soil nitrogen availability, as nitrogen is relevant to plant performance (Butler et al. 2012, Borer et al. 2014). For example, soil nitrogen levels may affect a plant's ability produce secondary defense compounds (Bryant et al. 1983, Ibrahim et al. 2011, De Long et al. 2015), or it may even change the nutrient content of a plant making it more or less appealing to herbivores (Waring and Cobb 1992, Oriens and Fritiz 1996,). The differing nutrient demands and growth rates between cytotypes likely influence herbivore feeding patterns (Nuismer and Thompson 2001, Thompson et al. 2004, Halverson et al. 2008, Hull-Sanders et al. 2009, Münzbergová et al. 2015); however, there are few studies explicitly testing the dynamics of soil nutrients and herbivory in polyploid systems (but see Bales (2015)).

To assess whether cytotype performance traits and insect-caused foliar damage are differentially influenced by the cytotype of a competitor plant based on nitrogen supplies, we conducted a greenhouse experiment using diploid and autotetraploid cytotypes of *C. angustifolium*. We grew diploid and autotetraploid fireweed within three different competition treatments (alone, with a diploid, with an autotetraploid) under two

nitrogen levels (low, high) to test whether the cytotype of a competitor plant influences diploid and tetraploids performance traits differently, and if these responses depend upon soil nitrogen supplies. Furthermore, we evaluated whether insects damage diploids and tetraploids differently under variable nitrogen conditions and if competition affects the feeding patterns of a generalist herbivore using a series of bioassays. Because diploids and tetraploids have different nutrient demands, we expect tetraploids to be more severely impacted by nitrogen scarcities; therefore, giving them a competitive disadvantage through the tradeoff of investing limited nitrogen supplies towards the creation of nucleic acids over investing in growth and photosynthesis.

1.3 Materials and Methods

Study organism:

Chamerion angustifolium, fireweed, is a circumboreal perennial, herbaceous plant that exists primarily as diploid ($2n=2x=36$ chromosomes per cell) and autotetraploid ($2n=4x=72$ chromosomes per cell) forms that are generally spatially segregated, although triploids do infrequently occur when diploid and autotetraploid ranges overlap in mixed ploidy populations (Mosquin 1967, Mosquin and Small 1971, Husband and Schemske 1998, Husband 2004). Diploid and tetraploid *C. angustifolium* differ in ecological and morphological traits that can contribute to competitive performance. For example, tetraploids are generally larger and flower earlier than diploids (Mosquin 1967, Husband and Schemske 1998, Husband 2000, Husband and Schemske 2000, Husband and Sabara 2004).

The lineages of *C. angustifolium* used in our study were originally collected from eight mixed-ploidy populations near Fairbanks, AK during the summers of 2013 and 2014, as a part of a study that evaluated the ploidy makeup of these populations (Bales 2015). The seeds from these populations were maintained in the greenhouse at Michigan Technological University (Department of Biological Sciences, Houghton, MI) under identical growing conditions. Ploidy level was verified with flow cytometry and 2C DNA contents for each genotype were compared to known standards (Bales 2015), and mature plants were cross-pollinated with individuals of the same cytotype to generate seeds for this study.

Experimental Design:

Greenhouse design and nitrogen treatments- In February 2017, we germinated 400 seeds (200 seeds from 10 diploid maternal lines and 200 seeds from 7 autotetraploid maternal lines) of *C. angustifolium* on damp filter paper in 60mm petri dishes under identical greenhouse conditions. Ten days after germination, seedlings were planted in a 1:1 mixture of Sungro Professional Potting Mix #2:Vermiculite (Sun Grow Horticulture Ltd., Vancouver, British Columbia) in 2L round plastic pots in one of three competition treatments: (1) alone (“no competition”), (2) with a seedling from a different genetic line but of the same ploidy level (“intra-cytotype competition”), or (3) with a seedling of a different ploidy level (“inter-cytotype competition”). Seedlings within competition treatments were planted in the same pot spaced 10.0 cm apart from each other and 5.0 cm from the wall of the pot, and pots in each competition treatment were divided into two soil nitrogen (N) levels: low or high.

Nitrogen was supplied to the plants as ammonium nitrate through four weekly dosages that totaled 10 ppm (low treatment) and 100 ppm (high treatment) of N per pot ($\mu\text{g N g}^{-1}$ soil). Low N concentrations were chosen based on average inorganic N levels ($\text{NH}_4\text{-N} + \text{NO}_3\text{-N}$) near the seed collection sites in interior Alaska near Fairbanks (GPS Coordinates: 64.70369 -148.29862) and similar boreal ecosystems (5-220 ppm N; Gordon et al. 1987, Clein and Schimel 1995, Stottlemeyer et al. 2003, Jerabkova et al. 2006, Bales 2015). Plants received excess phosphorus (P; 100 - 200 ppm supplied as potassium monophosphate), potassium (K; 250 ppm supplied as potassium sulfate), and

micronutrients (0.537 mL Fertlome chelated liquid iron and other micronutrients) per pot to supplement other macro- and micronutrients needed for plant growth. A combined N, P, and K solution was administered weekly to the plants during weeks four through seven of growth; the first two fertilizer treatments were diluted with 50% deionized water to avoid shocking the root system of young plants. A 50% dilute micronutrient solution was administered during the fifth week and a full solution during the tenth week. Plants were grown under a standard 16:8 hour light/dark cycle, and watered as needed until harvest at 17 weeks of growth; plants were also rotated weekly to minimize any effects from variable greenhouse conditions.

Performance Traits:

To assess the outcomes of plant competition we measured survival (yes/no), and ten performance traits related to growth and reproduction. For growth traits, we measured final plant height, aboveground, belowground, total biomass, and a plant's shoot:root ratio (S:R). To measure biomass, we harvested plants after flowering or at approximately 17 weeks if they showed no signs of flowering. Plants were severed at the soil line to separate above and belowground masses, dried at 60°C, and weighed each plant portion to the nearest gram. Shoots were dried for 48 hours, while roots were dried for 72 hours because roots contain more water and take longer to fully dry. Plants were weighed periodically throughout the weighing day to ensure they had fully dried to a consistent weight. From these values, we calculated the above- to belowground biomass ratio (S:R ratio) by dividing dried root weight by dried shoot weight. The size of a plant's above and belowground biomasses are reflective of its ability to gather resources and of the

trade-off between root and shoot growth based on external factors such as nutrient availability. For example, plants with a smaller S:R ratio may allocate more resources to root growth than shoot growth as a means to gather more water or nutrients from the soil. S:R ratios also play an important role in competitive dynamics as plants compete for resources with both their above and belowground biomasses and the allocation of growth towards these masses can influence competitive outcomes.

For reproductive traits, we measured flowering (yes/no), the number of flowers produced, seed production (self- and cross-pollination), and clonal root bud production. Flower production was measured as the number of fully bloomed flowers produced during the 17 weeks of growth. We assessed fruit production (yes/no) by performing hand-pollinated crosses. To do this we rubbed the pollen from two dehiscent anthers across stigma to simulate insect pollination for self- and cross-pollinations. Four flowers on each plant were selected for hand-pollination; all other flowers on the plant were sterilized by removing the stigma to prevent unwanted pollination. Self-pollinated flowers received pollen from a different flower on the same plant; while cross-pollinated flowers received pollen from different plants of the same cytotype within the same N treatment. Fruits were collected at maturity, seeds were cleaned, and the total number of seeds per pod were counted with a Pfueller Contador II seed counter (Pfueller GmbH, Kitzingen, Bavaria, Germany). We estimated potential maximum seed production (MSP) per self- and cross-pollination by multiplying the average number of seeds produced per each cross by the number of flowers present per plant. At harvest, we counted the number of developed root buds on a plant's root system, which is a form of asexual reproduction where new, clonal shoots develop from a plant's existing underground biomass.

Insect Herbivory Damage:

To evaluate if insect feeding patterns differ among diploids and tetraploids grown under different N supplies and competitive regimes, we conducted petri dish leaf-choice and whole plant choice bioassays. All bioassays used the generalist leaf-chewing herbivore *Spodoptera exigua* (Hübner) (Insecta: Lepidoptera: Noctuidae), beet armyworm that was reared from eggs at 29°C on provided food (insects and media from Benzon Research, Carlisle PA) until they reached the fourth instar stage. During the course of the experiment, plants were eaten by the common greenhouse pest *Frankliniella occidentalis*, Western flower thrip throughout the experiment, and we measured the amount of damage present on each plant.

Petri Dish Leaf Choice Bioassay- Leaves from both diploid and tetraploid plants grown alone under low or high N conditions were used in a total of 80 choice bioassays (40 low N assays, 40 high N assays). During the eleventh week of growth, a single leaf was taken from the upper portion of each plant and cut into a 3x1 cm² rectangle; we chose to use a standard size because variances in leaf size and shape can affect herbivore preference (Rivero-Lynch et al. 1996). Leaves were placed on either side of a 60mm petri dish lined with damp filter paper, and a single fourth instar *S. exigua* larvae was placed in the center of each dish. The dishes were sealed with Parafilm, and the insects were allowed to feed for 72 hours. After the feeding period, insects were removed, and the percentage of leaf area eaten was calculated by subtracted the area of post-feeding leaf scans from pre-feeding leaf scans using the software ImageJ (ImageJ V.1.48 software (Rasband, W.S., US National Institutes of Health, Bethesda, MD, USA)).

Whole Plant Assays- To evaluate whether nitrogen availability, focal and competitor ploidy level, and/or their interactions influence whole plant insect damage patterns, we selected 48 diploid and 48 tetraploid plants randomly from alone and inter-cyotype competition treatments grown under low and high N conditions (total = 96 plants). Next, during the twelfth week of growth, we measured the height of the plants, placed a single fourth instar larvae onto the soil in the center of each pot, sealed the plants with organza bags to prevent insect escape, and allowed the insects to feed for 96 hours. We removed the insects from the bags, and scored the whole-plant percent damage on an ordinal scale, where 0= 0%, 1= 1-5%, 2= 6-12%, 3= 13-20%, 4= 21-40%, 5= 41-60%, 6= 61-80%, 7= 80+%.

Damage from a Greenhouse Pest- *Frankliniella occidentalis*, damages plants by puncturing leaves and feeding on leaf contents, and by feeding on a flower's anther and stigma (Li et al. 2015). Before harvest, we measured the heights of all plants to use as a covariate and visually estimated the percentage of total leaf area damaged by *F. occidentalis* on an ordinal scale, where 0= 0%, 1= 1-5%, 2= 6-12%, 3= 13-20%, 4= 21-40%, 5= 41-60%, 6= 61-80%, 7= 80+%.

Statistical Analysis:

We ran a series of parametric and non-parametric statistical tests evaluating the effects of focal plant ploidy (diploid, tetraploid), competitor plant ploidy (alone, diploid, tetraploid), soil nitrogen (N) level (low, high), their interactions, and maternal genetic line (nested within focal ploidy) on performance traits. In the parametric analyses, maternal genetic line was treated as a random factor; otherwise, all other factors were

treated as fixed effects. Transformations to meet model assumptions were performed and noted when needed. All analyses were performed using JMP Pro 13 (SAS Institute, Cary, North Carolina, USA).

Performance Traits- 92 plants died in our experiment and we used a nominal logistic regression model to examine whether focal plant ploidy, competitor plant ploidy, and their interaction influenced a plant's chance of survival (yes/no). Many plants died prior to the addition of soil nitrogen treatments; therefore, nitrogen level was not included in this model. Next, using only the surviving plants, we used a full-factorial, mixed effects ANOVA models with restricted maximum likelihood (REML) estimators to examine whether focal plant, competitor plant, their interaction, and maternal genetic line effected five growth traits (final height, aboveground, belowground, and total biomass, and shoot:root ratio (log transformed). We ran these models separately for each nitrogen treatment to better interpret patterns among multiple factors (initially in a full model with nitrogen included, some three way interactions were significant) and because we were mainly interested in whether cytotypes differed in competitive abilities. Not all plants flowered during our experiment, and we used a nominal logistic regression model to examine whether focal plant ploidy, competitor plant ploidy, soil N level, and their interactions influenced a plant's likelihood of flowering (yes/no). Given that a plant successfully flowered, we ran full-factorial, mixed effects ANOVA models with a REML estimate by both low and high N treatments to test whether focal plant ploidy, competitor plant ploidy, their interaction, and maternal genetic line differently affected reproductive traits: flower production (square root transformed), maximum seed

production from self-crossed and out-crossed (both square root transformed) fruits, and root bud formation (square root transformed).

Herbivory- To determine whether cytotypes differed in insect feeding damage and whether feeding preference depended on N treatment, we used a full factorial, mixed effects ANOVA model with REML estimator under low and high nitrogen treatments on the percentage of leaf area consumed by *S. exigua* (log+1 transformed) during the petri dish leaf choice bioassays. We ran an ordinal logistic regression under both low and high N treatments to examine whether focal plant ploidy, competitor plant ploidy, their interaction, and maternal genetic line influenced the amount of leaf damage (ordinal metric) by *S. exigua* during the whole plant bioassays and the total amount of foliage damage caused by *F. occidentalis* (ordinal metric). Plant height was used as a covariate in both regression models.

1.4 Results

Performance Traits:

Focal plant ploidy and competitor plant ploidy both had a significant effect on the likelihood of focal plant survival, but the interaction among factors did not significantly affect the probability of survival (Table 1.1). Tetraploids were more likely to survive than diploids (88% of tetraploids survived versus 66% of diploids survived) and plants grown alone were more likely to survive than plants grown with a competitor (85.9% of plants grown alone survived versus 74.3% of those grown with a tetraploid and 71% of those grown with a diploid).

A competitor plant's ploidy significantly affected a focal plant's final height under both low and high nitrogen (N) conditions, but no other factor or interaction among factors significantly affected a focal plant's final height under either N treatments (Table 1.2 A.). Under low N treatments, plants grown with a tetraploid (LS Mean $\pm$ standard error, 24.51 cm $\pm$ 2.74 cm) were significantly shorter than plants grown with a diploid (34.88 cm $\pm$ 2.89 cm), which were significantly shorter than plants grown alone (43.21 cm $\pm$ 2.82 cm). Under high N treatments, plants growth with a tetraploid (31.04 cm $\pm$ 3.03 cm) and plants grown with a diploid (34.86 cm $\pm$ 3.09 cm) were both significantly shorter than plants grown alone (47.54 cm $\pm$ 3.06 cm).

Focal plant ploidy was only found to influence aboveground biomass in the low N treatment, but competitor plant ploidy affected aboveground biomass under both low and high N treatments; the interaction between factors was not significant under either N treatment (Table 1.2 B.). We only found a significant difference between the

aboveground biomass of diploids and tetraploids under low N treatments (Table 1.2 B.). Tetraploids were significantly heavier than diploids when grown in low nutrient treatments (tetraploids: $2.53 \text{ g} \pm 0.13 \text{ g}$, diploids: $1.79 \text{ g} \pm 0.14 \text{ g}$). Competitor plant ploidy significantly affected a plant's aboveground biomass. In both low and high N treatments, plants grown with a tetraploid were significantly lighter than those grown with a diploid, which were significantly lighter than plants grown alone (Figure 1.1 A-B).

Focal plant ploidy was also found to only influence belowground biomass accumulation in the low N treatment, but belowground biomass accumulation was found to be significantly influenced by competitor plant ploidy under both low and high N treatments; the interaction between factors was not significant under either N treatment (Table 1.2 C.). The belowground biomass of diploids and tetraploids only significantly differed under low N treatments, where tetraploids were larger than diploids (tetraploids $4.48 \text{ g} \pm 0.25 \text{ g}$, diploids: $2.23 \text{ g} \pm 0.24 \text{ g}$) (Table 1.2 C.). Similar to our findings for aboveground biomass, competitor plant ploidy significantly affected a plant's belowground biomass under both low and high N conditions (Table 1.2 C.). Under both low and high N treatments, plants grown with a tetraploid were significantly lighter than those grown with a diploid, which were significantly lighter than plants grown alone (Figure 1.1 C-D).

Total biomass was also influenced by focal plant ploidy under low N treatments, and by competitor plant ploidy under both low and high N treatments; the interaction between these factors was not found to be significant under low nor high N treatment (Table 1.2 D.) As seen with the aboveground and belowground biomasses, tetraploids were only significantly larger than diploids under low N treatments (tetraploids: $6.01 \text{ g} \pm$

0.34 g, diploids: $4.00 \text{ g} \pm 0.34 \text{ g}$) (Table 1.2 D.). Under both low and high N treatments, plants grown with a tetraploid (low: $2.73 \text{ g} \pm 0.33 \text{ g}$, high: $3.97 \text{ g} \pm 0.56 \text{ g}$) were significantly lighter than those grown with a diploid (low: $4.75 \text{ g} \pm 0.35 \text{ g}$, high: $5.62 \text{ g} \pm 0.56 \text{ g}$), which was significantly lighter than plants grown alone (low: $7.53 \text{ g} \pm 0.34 \text{ g}$, high: $8.69 \text{ g} \pm 0.57 \text{ g}$).

We did not detect any significant differences between focal plant ploidy, competitor plant ploidy, and their interaction on S:R ratio, regardless of soil N treatment (Table 1.2 E.)(Figure 1.1 E-F).

Reproductive Measures- Out of the 308 surviving plants, only thirty-seven percent of these plants produced a minimum of at least one flower. Competitor plant ploidy under both high and low N treatments had an effect on the probability of flowering, but no other factor or interaction among factors had a significant effect on the probability of flowering (Table 1.3). Under low N treatments, plants growing with a tetraploid were the least likely to flower overall; while under high N treatments, plants grown with either a diploid or a tetraploid were less likely to flower than those grown alone. However, of the plants that flowered, only competitor plant ploidy under high N conditions affected the number of flowers produced; no other factors or interactions among factors were significant (Table 1.4 A.). Under high N treatments, plants grown alone (5.39 ± 0.34) produced more flowers than plants grown with a tetraploid (4.16 ± 0.51); flower production of plants grown with a diploid (4.46 ± 0.49) did not differ from those grown alone or with a tetraploid. Maximum seed production from both self-pollinations and cross-pollinations

were not significantly influenced by focal plant ploidy, competitor plant ploidy, nor their interaction under either N treatment (Table 1.4 B-C.).

We found that focal plant ploidy and competitor plant ploidy significantly affected root bud production, and while these factors were not dependent on each other, they did depend upon N treatments (Table 1.4 D.). We found a significant difference between root bud production of diploids and tetraploids under high N treatments (Table 1.4 D.). Diploids produced significantly more root buds than tetraploids when grown in high N treatments (diploids: 2.40 ± 0.11 , tetraploids: 1.88 ± 0.11). Furthermore, we found that competitor plant ploidy significantly affected a plant's root bud production under both low and high N conditions (Table 1.4 D.). Under low nitrogen treatments, plants grown alone (2.31 ± 0.10) produced significantly more root buds than plants grown with a diploid (1.92 ± 0.11), which produced more than plants grown with a tetraploid (1.56 ± 0.10). Under high N treatments, only plants grown alone (2.46 ± 0.1) produced the most root buds (diploid: 2.05 ± 0.10 root buds, tetraploid: 1.92 ± 0.10 root buds).

Herbivory:

No main model factors were found to significantly influence insect feeding damage for either of the choice bioassays nor the final damage from *F. occidentalis* (Table 1.5, Table 1.6). However, we did find that *F. occidentalis* damaged relatively more foliage from taller plants and from certain maternal lines (Table 1.6).

1.5 Discussion

Morphological, physiological, and ecological differences between diploids and polyploids are thought to contribute to differences in competitive outcomes among cytotypes, to the likelihood of polyploid population establishment, and to cytotype spatial distribution patterns (Maherali et al. 2009, Manzaneda et al. 2012, Thompson et al. 2015). However, we know very little about how and which abiotic factors influence competitive and consumptive forces between diploid and closely related polyploids (Thompson et al. 2015). Here we tested the overall hypothesis that soil nitrogen availability would influence competitive dynamics between diploids and autotetraploid *C. angustifolium*. Specifically, we thought that the performance traits of tetraploids would be suppressed under low nutrient conditions due to their greater nitrogen demands relative to diploids, thus giving diploids a competitive advantage. Under high nitrogen conditions, we expected tetraploids to be relieved of this suppression and display more performance traits related to competitive ability. Additionally, we assessed whether nitrogen supply influenced insect foliar damage differently among diploids and tetraploids, and if being in competition with a plant of the opposite cytotype influenced these damage patterns. In general, we found that under both nitrogen treatments plant performance often depended on the ploidy level of a competitor plant. We also found that tetraploids were generally larger than diploids, especially when grown in low nitrogen treatments. Insect foliar damage was not influenced by a plant's ploidy level, a competitor plant's ploidy level, nor was nitrogen availability.

The cytotype of a competitor plant matters:

Competition in plants is regarded as the ability of a plant to suppress its neighbor's access to limited resources, such as water, sunlight, and soil nutrients (Aarssen 1983, Goldberg and Landa 1991, Craine and Dybzinski 2013), or avoid being suppressed by a neighboring plant (Goldberg and Fleetwood 1987, Goldberg and Landa 1991). Here we found that all plants experienced negative effects from competition as plants grown with another plant typically had smaller aboveground, belowground, and total biomasses in comparison to plants grown alone. Furthermore, we found that both diploids and tetraploids had smaller above-, below-, and total biomasses when grown with a tetraploid. This suggests that tetraploids have a greater suppressive effect on nearby plants than diploids.

Plant size is a key component to competitive interactions (Goldberg et al. 2017), as larger plants are better able to monopolize (Grime 1977) and suppress another plant's (Keddy et al. 2009) access to resources. We also found that tetraploids were generally bigger than diploids, and that this was especially prevalent in the low nitrogen treatment. We were surprised to find that neither focal plant ploidy or competitor plant ploidy influenced resource allocation strategies via a plant's shoot:root ratio. These strategies can have an impact on competitive interactions as plants mainly compete for resources such as sunlight and water through their aboveground and belowground biomasses, respectively (Aschehoug et al. 2016), and competitive stress can influence how these resources are allocated (Berendse and Moller 2009). However, we did not detect evidence that competitive stress changed resource allocation strategies in our experimental plants.

Upon further analysis of plant performance traits, we found that tetraploids had significantly larger aboveground biomass than diploids regardless of nitrogen treatment, and this finding is in agreement with other studies involving fireweed (Mosquin 1967, Burton and Husband 2000, Bales 2015, Thompson et al. 2015). Therefore, we expect that the observed effects of a tetraploid competitor on plant performance traits may have been influenced in part by aboveground biomass, thus implying that tetraploid fireweed competes via their aboveground structures most likely by shading smaller, neighboring plants (Grime 1977). The tetraploids plants in our study could have blocked its neighbor's access to sunlight, thus decreasing a neighboring plant's photosynthetic activity and biomass accumulation.

Traits associated with light competition, such as greater heights and biomasses, may aid in the structuring of mixed-ploidy populations and potential separation of diploid and polyploid populations in fireweed. Fireweed is an early successional species often associated with disturbed habitats, specifically post-fire disturbances (Fleenor 2016). Fireweed populations are commonly found in sunny environments, and studies have shown that their population densities decrease as over story shade increases, implying that fireweed may be shade intolerant (Lieffers and Stadt 1994). However, it is unknown if these traits are the direct result of polyploidization or if they have been selected for in polyploid populations over many generations. Evaluating which traits are an inherent and direct result of polyploidy and which are not will aid in our understanding of how polyploid populations successfully establish and structure themselves. The success and competitive ability of plants in different soil nutrient conditions can be attributed to their

resource allocation strategies and these strategies change when nutrient supplies are altered (Tilman and Cowan 1989).

Nitrogen supplies does not affect competition:

Nitrogen availability was found to be less impactful than expected, as most performance traits or trends related to performance traits typically did not differ under low and high nitrogen treatments regardless of a focal plant's ploidy or its competitor's ploidy. However, aboveground, belowground, and total biomass, as well as clonal root bud production did vary by focal cytotype under low and high nitrogen conditions. We were surprised to find that tetraploids plants were larger than diploid plants under low nitrogen treatments, as we had expected tetraploid performance traits to be suppressed due to the greater nutrient costs involved in synthesizing a polyploid genome. Our results are contrary to two recent field studies that found polyploid biomass to increase as soil nutrients increased (Smarda et al. 2013, Guignard et al. 2016). We are unsure why diploids, but not tetraploids, were limited by nitrogen scarcity, but speculate that genetic variation among our plants and/or the evolutionary selection for larger plant sizes in natural tetraploid populations may have influenced our findings.

We also found that diploids produced the most clonal root buds under high nitrogen conditions. This is particularly surprising to us, because the addition of nitrogen had no impact on tetraploid root bud production. In polyploids, asexual reproduction is considered especially advantageous after initial establishment (Whitton et al. 2008), and many studies have reported greater apomixis in polyploids relative to diploids (Eckert et al. 2003, Duchoslav and Staňková 2015, Herben et al. 2017). However, the benefits of

clonal growth are not limited to only polyploids. Diploids can also benefit from a clonal system by monopolizing and sharing underground resources through the growth of horizontal ramets (Gough et al. 2001). It is possible that diploids are more likely to use clonal growth in a competitive way to reduce a neighbor's access to underground resources; however, the short-term duration of our study did not allow us to test this idea further. Future studies could evaluate how abiotic factors differentially influence the clonal growth of diploids and polyploids, especially because of the important role clonal growth has on initial population establishment in polyploids.

Insect consumption patterns did not depend on ploidy level nor nitrogen supplies:

We did not find evidence that either focal plant or competitor plant ploidy levels influenced insect feeding damage, nor were these factors dependent on nitrogen supplies. However, other studies have found that a plant's ploidy level influences plant responses to herbivory and herbivore feeding patterns (Nuismer and Thompson 2001, Thompson et al. 2004, Halverson et al. 2008, Münzbergová et al. 2015). For example, Bales (2015) found that diploid fireweed were more resistant to *F. occidentalis* feeding than tetraploids, but that tetraploids were more resistant to *S. exigua* feeding.

Plant-herbivore interactions regulate and shape community dynamics through changes in plant fitness and competitive ability (Eskelinen et al. 2012, Borer et al. 2014), as plants may experience trade-offs in competitive ability through the physical damages and compensatory responses to herbivory (Borer et al. 2014). Soil nutrient availability also factors into these tradeoffs, as soil nutrients can be used to alleviate the consequences of herbivory (Lind et al. 2013, Borer et al. 2014) through the formation of

secondary defense compounds (Bryant et al. 1983, Ibrahim et al. 2011, De Long et al. 2015) or investment into rapid growth (Coley and Phyllis 1983). Anthropogenic increases in global nitrogen supplies are expected to have large-scale environmental consequences (Vitousek et al. 1997), such as the loss of plant diversity through the mitigation of competitive intensities among plants (Borer et al. 2014). Despite these known consequences, little is known about how soil nitrogen deposition can affect the plant-insect interactions shaping the structure of polyploid populations.

Conclusion:

The mechanisms resulting in polyploid establishment are not well understood, but it is theorized that competitive interactions between cytotypes play a key role (Levin 1975, Fowler and Levin 1984, Rodriguez 1996, Husband 2000). Because cytotypes exhibit differing morphological, physiological, and ecological tolerances, it is hypothesized that abiotic factors influence the competitive outcomes of diploids and polyploids differently, thus leading to spatial cytotype segregation and polyploid population establishment (Thompson et al. 2015). Overall, our findings suggest that competitive outcomes between diploids and tetraploids are influenced by the competitor's ploidy level, but not by nitrogen availability. We also found that nitrogen scarcity does not limit the growth, fitness, and resistance traits of tetraploids relative to diploids. Additionally, we recognize that genetic variability can also influence competitive outcomes as the variety and effectiveness of performance traits are associated with genetic diversity (Aarssen 1983, Aschehoug et al. 2016). Our findings contribute to the few studies testing the hypothesis that variable abiotic factors influence competitive

outcomes between cytotypes (but see Thompson et al. 2015), and it highlights the importance of evaluating how a plant's performance can be altered by the cytotype of its competitor. We conclude that competition does play an important role in polyploid population establishment and in cytotype spatial distribution patterns, as we observed a suppression in growth traits of plants grown with tetraploids.

Our understanding of how polyploids populations establish themselves would benefit from both greenhouse and field studies examining how the performance traits of diploids and polyploids differ under a variety of abiotic and biotic environmental factors. Additionally, studies examining whether traits related to polyploid competitive ability are a direct result of polyploidization or a result of evolutionary selection after polyploidization events would contribute to our understanding of polyploidy's role in angiosperm evolution.

1.6 Tables

Table 1.1: Results of two-way, fixed effects nominal logistic regression testing the effects of ploidy (diploid, tetraploid), competitor ploidy (alone, diploid, tetraploid), and their interaction on a plant's likelihood to survive. Nitrogen level was excluded from this analysis because most plants died before the nitrogen treatments were administered. Degrees of freedom (k-1, N-k) are reported, and bold values indicate a significant P -value at $\alpha = 0.05$.

Source	X^2_{df}	P
Focal Ploidy (FP)	25.712 _(1,398)	<0.0001
Competitor Ploidy (CP)	8.670 _(2,397)	0.0131
FP $\times$ CP	1.944 _(2,397)	0.3784

Table 1.2: Results of two-way, mixed-effects, full factorial ANOVAs with REML estimates testing focal plant ploidy (diploid, tetraploid), competitor plant ploidy (alone, diploid, tetraploid), their interactions on growth traits under low and high nitrogen treatments. Maternal genetic line was included in the models as a random effect. Degrees of freedom (k-1, N-k) are reported, and bold values indicate a significant *P*-value at $\alpha = 0.05$.

Factor	Source	Low Nitrogen		High Nitrogen	
		F _{df}	<i>P</i>	F _{df}	<i>P</i>
a. Final Height	Focal Ploidy (FP)	1.230 _(1,155)	0.2744	0.365 _(1,149)	0.5566
	Competitor Ploidy (CP)	23.812 _(2,154)	<0.0001	22.296 _(2,148)	<0.0001
	FP × CP	0.841 _(2, 154)	0.4332	0.798 _(2,148)	0.4524
b. Aboveground Biomass (g)	Focal Ploidy (FP)	15.360 _(1,155)	0.0032	2.612 _(1,149)	0.1145
	Competitor Ploidy (CP)	81.618 _(2,154)	<0.0001	57.255 _(2,148)	<0.0001
	FP × CP	1.168 _(2,154)	0.3137	1.900 _(2,148)	0.1534
c. Belowground Biomass (g)	Focal Ploidy (FP)	12.701 _(1,155)	0.0056	0.442 _(1,149)	0.5206
	Competitor Ploidy (CP)	46.313 _(2,154)	<0.0001	30.838 _(2,148)	<0.0001
	FP × CP	0.415 _(2,154)	0.6611	0.404 _(2,148)	0.6687
d. Total Biomass (g)	Focal Ploidy (FP)	17.951 _(1,155)	0.0024	0.871 _(1,149)	0.3723
	Competitor Ploidy (CP)	69.663 _(2,154)	<0.0001	52.058 _(2,148)	<0.0001
	FP × CP	0.784 _(2,154)	0.4582	0.958 _(2,148)	0.3860
e. Shoot:Root Ratio*	Focal Ploidy (FP)	1.452 _(1,155)	0.2570	0.384 _(1,149)	0.5595
	Competitor Ploidy (CP)	0.462 _(2,154)	0.6308	1.337 _(2,148)	0.2659
	FP × CP	0.650 _(2,154)	0.5235	0.542 _(2,148)	0.5830

* Factor log transformed

Table 1.3: Results of two-way, fixed effects nominal logistic regression testing the effects of ploidy (diploid, tetraploid), competitor plant ploidy (alone, diploid, tetraploid), and maternal genetic line on a plant's likelihood of flowering under low and high nitrogen treatments. Degrees of freedom (k-1, N-k) are reported, and bold values indicate a significant P -value at $\alpha = 0.05$.

Source	Low Nitrogen		High Nitrogen	
	X^2_{df}	P	X^2_{df}	P
Focal Ploidy (FP)	0.000014 _(1,155)	0.9970	0.000004 _(1,149)	0.9984
Competitor Ploidy (CP)	15.767 _(2,154)	0.0004	20.638 _(2,149)	<0.0001
FP $\times$ CP	1.957 _(2,154)	0.3748	0.910 _(2,149)	0.6346
Maternal Genetic Line	67.642 _(14,142)	<0.0001	74.400 _(14,136)	<0.0001

Table 1.4: Results of two-way, mixed-effects, full factorial ANOVAs with REML estimates testing focal plant ploidy (diploid, tetraploid), competitor plant ploidy (alone, diploid, tetraploid), their interactions on reproductive traits under low and high nitrogen treatments. Maternal genetic line was included in the models as a random effect. Degrees of freedom (k-1, N-k) are reported, and bold values indicate a significant P -value at $\alpha = 0.05$.

Factor	Source	Low Nitrogen		High Nitrogen	
		F _{df}	P	F _{df}	P
a. Number of Flowers*	Focal Ploidy (FP)	0.858 _(1,50)	0.3873	1.030 _(1,60)	0.3322
	Competitor Ploidy (CP)	1.109 _(2,49)	0.3391	3.265 _(2,59)	0.0459
	FP $\times$ CP	1.606 _(2,49)	0.2122	0.220 _(2,59)	0.8029
b. Maximum Seed Production: Self-Cross*	Focal Ploidy (FP)	0.933 _(1,46)	0.3620	1.015 _(1,56)	0.3321
	Competitor Ploidy (CP)	0.130 _(2,45)	0.8784	0.053 _(2,55)	0.9486
	FP $\times$ CP	0.512 _(2,45)	0.6042	0.298 _(2,55)	0.7438
c. Maximum Seed Production: Out-Cross*	Focal Ploidy (FP)	0.286 _(1,38)	0.6109	0.357 _(1,47)	0.5665
	Competitor Ploidy (CP)	1.902 _(2,37)	0.1658	0.778 _(2,46)	0.4660
	FP $\times$ CP	0.506 _(2,37)	0.6077	0.374 _(2,46)	0.6901
d. Number of Root Buds*	Focal Ploidy (FP)	0.047 _(1,155)	0.8334	11.121 _(1,149)	0.0169
	Competitor Ploidy (CP)	23.108 _(2,154)	<0.0001	13.718 _(2,148)	<0.0001
	FP $\times$ CP	2.440 _(2,154)	0.0907	0.733 _(2,148)	0.4821

* Factor square root transformed

Table 1.5: Results of the mixed-effects, full factorial ANOVA with REML estimates testing focal plant ploidy (diploid, tetraploid), nitrogen treatment (low, high), and their interaction on the percentage of leaf area consumed by *S. exigua* (log+1 transformed) during the petri dish bioassays. Maternal genetic line was included in the models as a random effect. Degrees of freedom (k-1, N-k) are reported, and bold values indicate a significant *P*-value at $\alpha = 0.05$.

Source	F _{df}	<i>P</i>
Focal Ploidy (FP)	2.940 _(1,160)	0.1306
Nitrogen (N)	2.903 _(1,160)	0.0905
FP × N	0.102 _(1,106)	0.7500

Table 1.6: Results of two-way, fixed effects ordinal logistic regression testing the effects of ploidy (diploid, tetraploid), competitor plant ploidy (alone, diploid, tetraploid), and maternal genetic line on insect feeding damage by *S. exigua* in the caged bioassays and by *F. occidentalis* at the end of the experiment. Plant height at the time of damage measurements was used as a covariate. Degrees of freedom (k-1, N-k) are reported, and bold values indicate a significant *P*-value at $\alpha=0.05$.

Factor	Source	Low Nitrogen		High Nitrogen	
		X^2_{df}	<i>P</i>	X^2_{df}	<i>P</i>
a. Leaf Area Consumed in Cage Bioassay	Focal Ploidy (FP)	0.534 _(1,47)	0.4648	2.765 _(1,43)	0.0964
	Competitor Ploidy (CP)	0.855 _(1,47)	0.3552	0.036 _(1,43)	0.8503
	FP × CP	0.154 _(1,47)	0.6946	2.330 _(1,43)	0.1214
	Maternal Genetic Line	7.126 _(11,37)	0.7888	16.142 _(12,32)	0.1848
	Height (cm)	0.055 _(1,47)	0.8153	2.452 _(1,43)	0.1174
b. Leaf Area Damaged by <i>F. occidentalis</i>	Focal Ploidy (FP)	2.099 _(1,155)	0.1474	0.332 _(1,149)	0.5646
	Competitor Ploidy (CP)	0.477 _(2,154)	0.7877	0.898 _(2,148)	0.6382
	FP × CP	0.272 _(2,154)	0.8728	3.616 _(2,148)	0.1639
	Maternal Genetic Line	32.636 _(14,142)	0.0033	13.106 _(14,136)	0.5182
	Height (cm)	5.368 _(1,155)	0.0205	1.030 _(1,149)	0.3102

1.7 Figures

Figure 1.1: The effects of competitor plant ploidy under low and high nutrient conditions on (A-B) aboveground biomass (low: $F_{(2,154)}=81.618$, $P < 0.0001$; high: $F_{(2,148)}=57.255$, $P < 0.0001$), (C-D) belowground biomass (low: $F_{(2,154)}= 46.313$, $P < 0.0001$; high: $F_{(2,148)}=30.383$, $P < 0.0001$), and (E-F) shoot:root ratio (low: $F_{(2,154)}=0.462$,

$P = 0.6308$; high: $F_{(2,148)}=0.5235$, $P = 0.5830$). Means $\pm$ SE (Shoot:Root log transformed). Different letters represent significantly different means from Tukey's post-hoc HSD test.

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Chapter 2: Evaluating the effects of nitrogen and phosphorus supplies and competitive interactions on the performance traits of diploid, established tetraploid, and neotetraploid *Chamerion angustifolium*

2.1 Abstract

Newly formed polyploids (neopolyploids) in diploid-dominant populations face strong barriers preventing their establishment; however, established polyploid populations are prevalent in angiosperm species. Differences in competitive abilities brought on by genome duplication can vary with abiotic and biotic environmental conditions and influence polyploid establishment. Here we test the hypothesis that soil nutrient availability affects competitive outcomes among diploids and tetraploids. We grew diploid, autotetraploid, and second generation neotetraploid cytotypes of *Chamerion angustifolium* alone or with each other under different nitrogen and phosphorus concentrations to examine plant performance traits. We found that whole genome duplication affects competitive outcomes, but these outcomes vary depending on nutrient supplies. We also examined if foliar damage from feeding insects differed between cytotypes, was influenced by a competing plant's cytotype, and depended upon soil nutrient supplies. Whole genome duplication also influenced foliar insect damage, and this damage varied based on soil nutrients. We did not find evidence of neopolyploids being superior competitors as they were the most damaged by herbivores, least likely to flower, and most likely to die before reaching maturity. Our results suggest that soil

nutrient availability can influence competitive outcomes between diploids and polyploids, potentially having a key role in the mechanisms leading to polyploid population establishment. Evaluating how other abiotic and biotic factors influence performance traits and subsequent competitive abilities among cytotypes will aid in our understanding of polyploidy's ecological role.

2.2 Introduction

Polyploidy, or whole genome duplication, is a mechanism of diversity and evolution in flowering plants (Soltis & Soltis 1999, Husband 2000, Soltis & Soltis 2009) that results in the immediate accumulation of genetic diversity within species (Otto & Whitton 2000). Polyploidization can influence the way plants interact with their abiotic and biotic environments (Segraves 2017) through changes in morphological traits that influence ecological tolerances (Soltis and Soltis 1999, Thompson et al. 2014, Thompson et al. 2015) such as water and nutrient demands (Lewis 1985, Leitch and Bennett 2004, Cavalier-Smith 2005, Thompson et al. 2014, Guignard et al. 2017). Upon formation, neopolyploids (early generation polyploids) are vastly outnumbered by related diploids and may have difficulties finding mates, as polyploid-diploid offspring are usually inviable or sterile due to their differing genome sizes (Levin 1975, Husband and Schemske 2000, te Beest et al. 2012). However, polyploids may successfully establish viable populations under ideal conditions, if they are able to: colonize a niche distinct from progenitor diploids (Fowler and Levin 1984, Maceira et al. 1993, Rodriguez 1996, Husband and Sabara 2004), outcompete diploids for resources (Rodriguez 1996, Husband 2000, te Beest et al. 2012, Rey et al. 2017), and reproduce asexually via self-compatibility and clonal growth (Otton and Whitton 2000, Van Drunen and Husband 2018). Competition between cytotypes (plants of the same species with different genome sizes) and differences in herbivore consumption patterns likely influence neopolyploid success, as both forces are instrumental in shaping plant community structure (Grime 1977, Grime and Hodgson 1987, Crawley 1989, Fraser and Keddy 2005, Eskelinen et al.

2012, Kempel et al. 2015). Differences in abiotic and biotic environmental factors could influence competitive interactions and plant-herbivore dynamics between cytotypes (Collins et al. 2011, Borer et al. 2014, Thompson et al. 2015). However, the processes by which these factors interact to establish independent polyploid populations and define cytotype distributions are still largely unknown (Collins et al. 2011, Thompson et al. 2015). The main goal of our study was to determine if variation in soil nitrogen and phosphorus concentrations influence the competitive outcomes between diploid, established tetraploid, and neotetraploid fireweed (*Chamerion angustifolium*) as an explanation for the evolutionary processes that affect polyploid population establishment. We also examined whether these variations in nitrogen and phosphorus availability influenced insect foliar damage differentially between diploids, established tetraploids, and neotetraploids.

Neopolyploids are early generation polyploids that have had little exposure to selective forces, and are excellent models for comparing innate versus evolved traits in polyploid complexes (Ramsey and Schemske 2002). Neotetraploids share their genomic information with parental diploids, and studies comparing these cytotypes allow us to discern the phenotypic effects of whole genome duplication, while studies comparing neopolyploids to established polyploids can reveal the effects of evolution and adaptation on the phenological traits (Ramsey and Schemske 2002, De Kovel and De Jung 2000, Ramsey 2011). However, the exact ecological and evolutionary mechanisms aiding in polyploid establishment are still largely unknown, despite the prevalence of polyploid populations in angiosperm species. Polyploidization sometimes results in disadvantages

that hinder the chances of establishment, such as irregularities in chromosomal separation (Comai 2005, Cifuentes et al. 2010) that may inhibit sexual reproduction.

Polyploidization can also result in morphological differences between diploids and established polyploids (Burton and Husband 2000, Otto and Whitton 2000, Nuismer et al. 2005, Segraves 2017), which could aid in establishment. Furthermore, recent studies have shown that neopolyploids also display phenotypic divergence from their diploid progenitors (Ramsey 2007, Oswald and Nuismer 2010, Martin and Husband 2012, Baldwin and Husband 2013, Van Drunen and Husband 2018), implying that these differences could facilitate neopolyploid survival and establishment, thus playing a key role in the competition and consumption forces known to shape plant communities (Grime 1977, Grime and Hodgson 1987, Crawley 1989, Fraser and Keddy 2005, Eskelinen et al. 2012, Kempel et al. 2015).

In plants, competition between individuals (Grime 1977, Grime and Hodgson 1987, Goldberg and Barton 1992, Fraser and Keddy 2005) and herbivore consumption patterns (Crawley 1989, Maron and Crone 2006, Eskelinen et al. 2012, Borer et al. 2014, Kempel et al. 2015) are two of the most important mechanisms shaping plant population and community structure. Because plants are immobile and only require a few, key resources (i.e. light, water, soil nutrients), competitive outcomes are largely based upon a plant's ability to suppress its neighbor's access to resources (Aarssen 1983, Goldberg and Landa 1991, Craine and Dybzinski 2013) or prevent itself from being suppressed (Goldberg and Fleetwood 1987, Goldberg and Landa 1991). Traits such as aboveground biomass (Goldberg et al. 2017), belowground biomass (Aerts 1999), and growth rate (Coley 1983) are reflective of a plant's competitive ability, and variation in these traits as

well as how a plant allocates resources towards these traits are heavily influenced by both biotic and abiotic factors (Brooker et al. 2005, Chamberlain et al. 2014, Aschehoug et al. 2016), as well as genetic diversity (Aarssen 1983, Aschehoug et al. 2016) and ontogeny (Schiffers and Tielborger 2006). Plant-herbivore interactions can change a plant's overall fitness and competitive ability (Crawley 1989, Eskelinen et al. 2012, Borer et al. 2014), as plants may experience trade-offs that effect competitive traits through the physical damages caused by herbivory and compensatory responses a plant undergoes to maintain its survival and fitness (Borer et al. 2014). For example, foliar feeding can reduce a plant's photosynthetic activity as well as its ability to block its neighbors access to sunlight, thus resulting in competitive disadvantage in comparison to an undamaged neighbor (Crawley 1989). Competitive and consumption forces could be especially influential in polyploid establishment because the physiological and morphological differences between diploids and polyploids are thought to influence competitive outcomes (Maceria et al. 1993, Sugiyama 1998, Baack and Stanton 2005, Münzbergová et al. 2007, Maherali et al. 2009, Fialova and Duchoslav 2014, Thompson et al. 2014, Thompson et al. 2015) and insect feeding preferences (Nuismer and Thompson 2001, Thompson et al. 2004, Halverson et al. 2008, Hull-Sanders et al. 2009, Münzbergová et al. 2015). However, these forces are often influenced by environmental factors (Dudt and Shure 1994, Aerts 1999, Bale et al. 2002), and because some diploids and polyploids differ in their ecological tolerances, abiotic environmental factors could have an important role in shaping polyploid population structure.

Here we evaluate the hypothesis that variable nitrogen and phosphorus supplies differentially effect the competitive performance of diploid, established tetraploid, and

neotetraploid cytotypes of fireweed, *Chamerion angustifolium* (L.) (Onagraceae). Plants require nutrients for photosynthesis and growth (Aerts 1999), and nitrogen and phosphorus are important, but limited, nutrients for plants (Turitzin 1982, Elser et al. 2007, Agren 2012). Both elements play an essential role in the structuring of DNA, RNA, and proteins necessary for the regulation of genetic mechanisms and vital cellular functioning. Nitrogen is especially important in the structural formation of proteins and nucleic acids, and plays a key role in catalyzing the light reactions in photosynthesis (Terry et al. 1985, Evans 1989, Berdalet et al. 1994, Leghari et al. 2016). Through its structural role in DNA and RNA, phosphorus regulates protein synthesis (Blevins 1999). Phosphorus is also the main reactive component to ATP and ADP, both of which are vital to cellular metabolism and photosynthesis (Blevins 1999). Despite their necessity in plant survival, both nitrogen and phosphorus tend to be limited resources in most ecosystems (Elser et al. 2007, Agren 2012) as both must be converted to a usable form by microorganisms and often leach from soils into bodies of water. Polyploids have larger genomes than diploids; therefore, they are hypothesized to require more nitrogen and phosphorus to build the molecular structure of their chromosomes (Leitch and Bennett 2004, Cavalier-Smith 2005, Guignard et al. 2017). Limitations in soil nutrient availability are expected to have a negative effect on polyploids in regard to their growth and reproductive traits, competitive ability, and herbivore interactions (Leitch and Bennett 2004, Cavalier-Smith 2005, Bales 2015, Guignard et al. 2017). For example, recent studies have found that polyploid plants perform better when grown in nitrogen and phosphorus rich soils (Smarda et al. 2013, Guignard et al. 2016). Therefore, soil nutrient availability is theorized to be a major abiotic factor influencing the competitive outcomes

between cytotypes as well as the tradeoffs used by plants to alleviate the consequences of herbivory.

We conducted a greenhouse experiment using diploid, established tetraploid, and neotetraploid *C. angustifolium* to assess if a cytotype's performance traits (growth and reproduction) and the amount of insect foliar damage are differentially influenced by a competing plant's cytotype under low and high nutrient supplies. Cytotypes were grown within four competition treatments (alone, with a diploid, established tetraploid, or neotetraploid) under two nitrogen:phosphorus nutrient treatments (low, high) to test if a competitor's cytotype influences performance traits and if these responses depend upon soil nutrient supplies. We also tested if the amount of foliar insect damage differs between diploids, established tetraploids, and neotetraploids and if it was dependent upon the cytotype of a plant's competitor and/or soil nutrient supplies through a series of choice bioassays using a generalist herbivore. Because both established and neotetraploids have a greater nutrient demand relative to diploids, we expected them to perform poorly under low nutrient conditions, therefore inhibiting their competitive performance and lessening the competitive effect they would have on a neighboring plant. We expected the opposite to be true of diploids grown in low nutrient conditions: where their competitive ability would not be influenced by nutrient limitations, therefore having a stronger competitive presence over neighboring plants.

2.3 Materials and Methods

Study Organism:

Chamerion angustifolium (L.) Holub (Onagraceae), fireweed, is a circumboreal, perennial forb of northern temperate latitudes. Diploid ($2n=2x=36$ chromosomes per cell) and autotetraploid ($2n=4x=72$ chromosomes per cell) cytotypes occur and usually vary in spatial distributions and ecological tolerances, but mixed populations do occasionally occur and produce relatively uncommon triploids (Mosquin and Small 1971, Husband and Schemske 1998, 2000). Diploid and autotetraploid *C. angustifolium* are known to differ in ecological tolerances and morphological traits that could influence competitive outcomes. For example, tetraploid plants are generally larger plants and produce larger, but fewer seeds than diploids (Mosquin 1967, Husband and Schemske 1998, Husband 2000, Husband and Schemske 2000).

During the summers of 2013 and 2014, the Hersch-Green Lab collected *C. angustifolium* seeds from eight mixed-ploidy populations of *C. angustifolium* near Fairbanks, AK to evaluate the ploidy makeup of these populations. Seeds were maintained by hand-pollinated out-crosses between cytotypes in a controlled greenhouse at Michigan Technological University (Department of Biological Sciences, Houghton, MI) under identical conditions for two generations. The ploidy level of all plants was verified by estimating plant nuclear 2C DNA content using flow cytometry to compare *C. angustifolium* DNA content to the known standards (Bales 2015). After all ploidy levels were verified, mature plants were cross-pollinated with an individual of the same

cytotype to create known genetic lines of diploids and established tetraploids for this study.

To obtain neopolyploids, diploid seeds from our greenhouse-maintained, known genetic lines were treated with colchicine to induce polyploidization following an adaptation of the methods described in Baldwin and Husband (2011) by the Hersch-Green lab in 2014. Seedlings were sown under identical conditions and treated with a 20 μ L drop of 0.02% colchicine balanced between their cotyledons and replenished as needed. The new ploidy level of all colchicine-treated plants was verified with flow cytometry, and plants that had successfully undergone polyploidization were cross-pollinated over two generations to generate neopolyploid seeds used in this experiment.

Experimental Design:

Greenhouse design and nutrient treatments- In February 2018, we germinated 420 seeds (140 diploids, established autotetraploids, and neotetraploids from 7 maternal lines of each cytotype) of *Chamerion angustifolium* on damp filter paper in 60mm petri dishes under identical greenhouse conditions. Ten days after germination, seedlings were planted in a 1:1 mixture of Sungro Professional Potting Mix #4:Vermiculite (Sun Grow Horticulture Ltd., Vancouver, British Columbia) in 2L round plastic pots in one of three competition treatments: (1) alone (“no competition”), (2) in a pot with a seedling of the same ploidy variant (“intra-cytotype competition”), or (3) in pots with either of the two remaining ploidy variants (“inter-cytotype competition”). Seedlings within competition treatments were spaced 10.0 cm from each other and 5.0 cm away from the edge of the

pot. Pots within each competition treatments were then divided into two soil nutrient treatments: low or high.

Nitrogen (N) and phosphorus (P) was administered to plants through four dosages throughout the experiment as ammonium nitrate and potassium phosphate, respectively. Low nutrient concentrations (total N: 10 ppm ($\mu\text{g N g}^{-1}$ soil) and total P: 1 ppm ($\mu\text{g P g}^{-1}$ soil) per pot). We chose our low nutrient concentrations based upon reported values for N and P near our seed collection sites in interior Alaska and similar boreal ecosystems (Gordon et al. 1987, Dyrness et al. 1989, Yarie 1991, Clein and Schimel 1995, Stottlemeyer et al. 2003, Neff et al. 2005, Jerabkova et al. 2006, Bales 2015). High nutrient concentrations (total N: 200 ppm ($\mu\text{g N g}^{-1}$ soil) and total P: 20 ppm ($\mu\text{g P g}^{-1}$ soil) per pot) were chosen to mimic naturally occurring nutrient values while keeping the Redfield ratio (N:P = 16:1). Plants also received potassium (K; 250ppm, supplied as potassium sulfate) and micronutrients (0.615 mL Fertilome chelated liquid iron and other micronutrients) per pot. A combined N, P, and K solution was administered weekly to the plants during weeks five through eight of growth; this timeframe was chosen because we noticed that in our previous greenhouse studies seedling mortality was highest prior to week five. The first two fertilizer treatments were diluted with 50% deionized water to avoid shocking the root system of young plants, and a 50% dilute micronutrient solution was administered during the fifth week while a full solution was administered during the ninth week. For 15 weeks, plants were grown under a standard 16:8 hour light/dark cycle, and watered as needed until harvest; we also rotated all plants weekly to reduce any random effects from variable greenhouse conditions.

Performance Traits:

We measured plant survival (yes/no), and seven performance traits related to growth and reproduction to assess the outcomes of cytotype competition under low and high nutrient supplies. For growth traits, we measured final plant height, final above-, below-, and total biomass, and each plant's shoot:root ratio (S:R). We measured biomass by harvesting all plants after they ceased flower production or at approximately 15 weeks of growth. Plants were severed at the soil line to separate the biomasses into aboveground and belowground portions, dried at 60°C, and weighed to the nearest gram once completely dry. We dried the aboveground biomasses for 48 hours, and the belowground for 72 hours because plants store more water in their root systems resulting in longer drying times. Plants were periodically weighed through their weighing day to ensure they had dried to a consistent, final weight. We calculated the shoot:root ratio (S:R) by dividing a plant's belowground biomass weight by its aboveground biomass weight. Larger numbers are indicative of a plant investing more resources into its shoot system, rather than its root system. The relative size of a plant's shoot system, relative to its root system, is reflective of its ability to gather and allocate resources, as well as its response and tradeoffs under variable external factors. Resource allocation patterns also play an important role in competitive dynamics, as plants utilize both their above- and belowground biomasses to compete for limited resources such as water, sunlight, and soil nutrients.

For reproductive traits, we measured flowering (yes/no), the number of flowers produced, seed production (self- and cross-pollination), and clonal root bud production.

We first counted the number of fully bloomed flowers produced by a plant throughout the course of the experiment. Fruit and seed production was assessed by performing hand-pollinated crosses in which we rubbed the pollen from two dehiscent anthers across stigma to mimic insect pollination. We selected four flowers per plant to hand-pollinate; all other flowers sterilized by removing the stigma. Self-pollinated flowers received pollen from a different flower on the same plant, and cross-pollinated flowers received pollen from a different plant of the same ploidy level grown within the same nutrient treatment. Once fruits matured, they were collected and cleaned. The number of seeds produced per pod is pending analysis, and will be counted with a Pfueller Contador II seed counter (Pfueller GmbH, Kitzingen, Bavaria, Germany). At harvest, we counted the number of root buds on a plant's root system, which is a form of asexual reproduction through the production of new, clonal shoots that develop from a plant's belowground biomass.

Insect Herbivory Damage:

To assess whether insect feeding patterns differ among cytotypes and whether these patterns are influenced by a competitor plant's cytotype and/or soil nutrient supplies, we conducted petri dish leaf-choice and whole-plant choice bioassays. All bioassays used *Spodoptera exigua* (Hübner) (Insecta: Lepidoptera: Noctuidae), a generalist leaf-chewing insect that we reared from eggs at 29°C on provided food (insects and media from Benzon Research, Carlisle PA) until they reached the fourth instar stage. Throughout the course of our experiment, we noticed that our plants were being eaten by

the common greenhouse pest *Frankliniella occidentalis* (Western flower thrip), and we measured the amount of damage present on each plant as additional data.

Petri Dish Leaf Choice Bioassay- Leaves from randomly selected diploid, established tetraploid, and neotetraploid plants grown under low or high nutrient conditions were used in a total of 80 bioassays (40 low nutrient and 40 high nutrient). During the twelfth week of growth, we took a single leaf from the upper portion of each plant and cut it into a standard size of a 3x1cm² rectangle. Leaf size was standardized because differences in leaf size and shape can affect herbivore preference (Rivero-Lynch et al. 1996). Leaves were placed equidistant from each other in a 60mm petri dish lined with damp filter paper to prevent leaves from drying out, and a single fourth instar *S. exigua* larvae was placed in the center of each dish. Each dish was sealed with Parafilm and the insects were allowed to feed for 72 hours. After the feeding period, we removed each insect and calculated the percentage of area eaten on each leaf by subtracting the area of post-feeding leaf scans from digitally recreated pre-feeding leaf scans using the software ImageJ (ImageJ V.1.48 software (Rasband, W.S., US National Institutes of Health, Bethesda, MD, USA)).

Whole-Plant Choice Cage Bioassays- To evaluate whether insect feeding damage is influenced by the cytotype of a competitor plant, and if they are dependent upon soil nutrients, we selected a total of 115 pots (see Appendix 1 for specific treatment sample sizes) to use in whole-plant choice bioassays. During the twelfth week of growth, we measured the height of selected plants, placed two fourth instar larvae in the center of each pot, and allowed to feed for five days. All plants containing insects were sealed with

organza bags to prevent the insects from escaping, and as a control, we also bagged 50 additional, random plants to account for any effects the organza bags may have on plant performance. Once insects were removed from the plants, we scored the whole-plant percent damage on an ordinal scale, where 0= 0%, 1= 1-5%, 2= 6-12%, 3= 13-20%, 4= 21-40%, 5= 41-60%, 6= 61-80%, 7= 80+%.

Damage from a Greenhouse Pest: *Frankliniella occidentalis* is a puncturing insect that feeds on the contents inside of leaf tissues; *F. occidentalis* also feeds on anthers and stigmas, often leaving flowers sterilized (Li et al. 2015). At harvest, we visually estimated the percentage of a plant's total leaf area damaged by *F. occidentalis* on an ordinal scale, where 0= 0%, 1= 1-5%, 2= 6-12%, 3= 13-20%, 4= 21-40%, 5= 41-60%, 6= 61-80%, 7= 80+%. We also measured a plant's final height at this time to be used as a covariate in subsequent statistical analyses.

Statistical Analyses:

We ran a series of parametric and non-parametric statistical tests evaluating the effects of focal plant ploidy (diploid, established tetraploid, neotetraploid), competitor plant ploidy (alone, diploid, established tetraploid, neotetraploid), soil nutrient level (low, high), their interactions, and maternal genetic line (nested within focal ploidy) on performance traits. In the parametric analyses, maternal genetic line was treated as a random factor; otherwise, all other factors were treated as fixed effects. Transformations to meet model assumptions were performed and noted when needed. All analyses were performed using JMP Pro 13 (SAS Institute, Cary, North Carolina, USA).

Performance Traits- 85 plants died in our experiment and we used a nominal logistic regression model to examine if focal plant ploidy influenced a plant's chance of survival (yes/no). Many plants died prior to the addition of soil nutrient treatments and before plants were considered large enough to compete with each other; therefore, nutrient level and competitor plant ploidy was not included in this model. Next, using only the surviving plants, we used a full-factorial, mixed effects ANOVA models with restricted maximum likelihood (REML) estimators to examine whether focal plant, competitor plant, their interaction, and maternal genetic line effected five growth traits (final height, aboveground, belowground, and total biomass (all square root transformed), and shoot:root ratio (log transformed). We ran these models separately for each nutrient treatment to better interpret patterns among multiple factors (initially in a full model with nutrients included, most three way interactions were significant, see Appendix 2) and because we were mainly interested in whether cytotypes differed in competitive abilities. Not all plants flowered during our experiment, and we used a nominal logistic regression model to examine whether focal plant ploidy, competitor plant ploidy, and their interaction influenced a plant's likelihood of flowering (yes/no) differently under low and high nutrient supplies. Given that a plant successfully flowered, we ran a full-factorial, mixed effects ANOVA model with a REML estimate by both low and high nutrient treatments to test whether focal plant ploidy, competitor plant ploidy, their interaction, and maternal genetic line differently affected reproductive traits: flower production (square root transformed), and root bud formation (square root transformed).

Herbivory- To determine whether cytotypes differed in insect feeding damage and whether the damage depended on nutrient treatment, we used a full factorial, mixed

effects ANOVA model with REML estimator under low and high nutrient treatments on the percentage of leaf area consumed by *S. exigua* (square root transformed) during the petri dish leaf choice bioassays. We ran an ordinal logistic regression under both low and high nutrient treatments to examine whether focal plant ploidy, competitor plant ploidy, their interaction, and maternal genetic line influenced the amount of leaf damage (ordinal metric) by *S. exigua* during the whole plant bioassays and the total amount of foliage damage caused by *F. occidentalis* (ordinal metric). Plant height was used as a covariate in both regression models.

2.4 Results

Performance Traits:

Growth Traits- During the first five weeks of growth, neotetraploids were significantly more likely to die than diploids or tetraploids ($X^2_{df=2} = 41.119$; $P < 0.0001$). For example, 58.6% of neotetraploids survived as opposed to 82.9% of diploids and 90.7% of tetraploids. After five weeks of growth, there was a minimal number of plant deaths.

The three-way interaction between focal plant ploidy, competitor plant ploidy, and soil nutrient treatment were significant for final plant height, aboveground biomass, total biomass, and root bud production (Appendix 2), so we opted to run 2-way analyses under both nutrient conditions and used Tukey's HSD post-hoc test within a focal cytotype to interpret our results. Focal plant ploidy was only found to influence the final height of a plant under low nutrient conditions, while a competitor plant's ploidy affected final height under high nutrient conditions (Table 2.1 A.). Under both low and high nutrient conditions, the interaction of focal plant ploidy $\times$ competitor plant ploidy significantly affected plant height (Table 2.1 A.). Under low nutrients, diploids (LS Mean $\pm$ standard error; 40.69 cm $\pm$ 4.125 cm) were significantly taller than neotetraploids (22.12 cm $\pm$ 4.765 cm), but neither differed from established tetraploid height (26.93 cm $\pm$ 3.990 cm). Under high nutrients, plants grown alone (48.36 cm $\pm$ 2.442 cm) were significantly taller than those grown with a diploid (37.82 cm $\pm$ 3.330 cm), but neither differed from plants grown with an established tetraploid (38.811 cm $\pm$ 3.043 cm) or a neotetraploid (48.60 cm $\pm$ 3.611 cm). The cytotype of a competitor influenced final plant height in diploids and established tetraploids, but not in established tetraploids, and the

effect of a competitor cytotype on final height was dependent on soil nutrient supplies. For diploids growing in low nutrient conditions, those growing with another diploid plant ($15.84 \text{ cm} \pm 5.900 \text{ cm}$) were significantly shorter than those growing alone ($49.09 \text{ cm} \pm 4.351 \text{ cm}$), with an established tetraploid ($44.33 \pm 7.190 \text{ cm}$), or with a neotetraploid ($57.17 \text{ cm} \pm 10.026 \text{ cm}$). Under high nutrient conditions, diploids growing with another diploid ($32.84 \text{ cm} \pm 4.190 \text{ cm}$) were significantly shorter than diploids growing alone ($47.81 \text{ cm} \pm 3.206 \text{ cm}$) or with an established tetraploid ($55.26 \text{ cm} \pm 4.866 \text{ cm}$), but none of these competition regimes differed from diploids growing with a neotetraploid ($34.72 \pm 6.583 \text{ cm}$). Established tetraploids growing under low nutrient conditions were shortest when growing with another established tetraploids ($17.63 \text{ cm} \pm 5.732 \text{ cm}$) and tallest when growing with a diploid ($36.93 \text{ cm} \pm 7.220 \text{ cm}$), but neither of these competition regimes differed from established tetraploids growing alone ($27.17 \text{ cm} \pm 6.390 \text{ cm}$) or with a neotetraploid ($27.67 \text{ cm} \pm 8.119 \text{ cm}$). Under high nutrient conditions, established tetraploids growing with another established tetraploid ($29.20 \text{ cm} \pm 4.498 \text{ cm}$) were significantly shorter than established tetraploids growing alone ($46.49 \text{ cm} \pm 4.416 \text{ cm}$), with a diploid ($47.58 \text{ cm} \pm 5.534 \text{ cm}$), or with a neotetraploid ($62.98 \text{ cm} \pm 6.504 \text{ cm}$). Neotetraploid height was not significantly influenced by competitor plant ploidy under either of the nutrient conditions.

Focal plant ploidy level was only found to influence aboveground biomass under high nutrient conditions, but a competitor's ploidy level and the interaction between focal plant ploidy and competitor plant ploidy were both significant under low and high nutrient conditions (Table 2.1 B.) Established tetraploids (Square root transformed LS Mean $\pm$ standard error; $1.87 \text{ g} \pm 0.083 \text{ g}$) had significantly heavier aboveground

biomasses than diploids ($1.37 \text{ g} \pm 0.089 \text{ g}$) and neotetraploids ($1.46 \text{ g} \pm 0.102 \text{ g}$) under high nutrient conditions. Under low nutrient conditions, plants growing with a diploid ($0.77 \text{ g} \pm 0.129 \text{ g}$) had the lowest aboveground biomass, while those growing alone ($1.18 \text{ g} \pm 0.097 \text{ g}$) or with a neotetraploid ($1.28 \text{ g} \pm 0.138 \text{ g}$) had the highest, but neither competition regime differed from plants growing with an established tetraploid ($0.93 \text{ g} \pm 0.117 \text{ g}$). However, under high nutrient conditions, plants growing with an established tetraploid ($1.21 \text{ g} \pm 0.096 \text{ g}$) had lower aboveground biomasses than those growing alone ($1.89 \text{ g} \pm 0.079 \text{ g}$) or with a neotetraploid ($1.74 \text{ g} \pm 0.114 \text{ g}$), but plants growing with an established tetraploid did not differ from those growing with a diploid ($1.42 \text{ g} \pm 0.105 \text{ g}$). The cytotype of a competitor influenced aboveground biomass accumulation for only diploids under low nutrient conditions (Figure 2.1 A); however, under high nutrient conditions, the cytotype of a competitor influenced aboveground biomass accumulation for diploids, established tetraploids, and neotetraploids (Figure 2.1 B.). Diploids grown with another diploid under low nutrient conditions were significantly smaller than those grown alone or with a neotetraploid (Figure 2.1 A.). Under high nutrient conditions, both established tetraploids and neotetraploids grown with an established tetraploid had smaller aboveground biomasses, as did diploids grown with another diploid (Figure 2.1 B.)

Focal plant ploidy, competitor plant ploidy, and the interaction among these factors significantly influenced belowground biomass and were depended upon soil nutrient treatment (Table 2.1 C.). Under low nutrient conditions, diploids (Square root transformed LS Mean $\pm$ standard error; $1.10 \text{ g} \pm 0.107 \text{ g}$) were only significantly heavier than neotetraploids ($0.66 \text{ g} \pm 0.123 \text{ g}$). Established tetraploids ($1.46 \text{ g} \pm 0.077 \text{ g}$) were

significantly heavier than diploids ($1.15 \text{ g} \pm 0.082 \text{ g}$) under high nutrient conditions. In low nutrient conditions, plants growing with a diploid ($0.70 \text{ g} \pm 0.113 \text{ g}$) had the smallest belowground biomass, while those growing with a neotetraploid had the largest ($1.11 \text{ g} \pm 0.121 \text{ g}$); but neither of these competition regimes differed from plants growing alone ($0.99 \text{ g} \pm 0.086 \text{ g}$) or with an established tetraploid ($0.85 \text{ g} \pm 0.103 \text{ g}$). Plants growing with an established tetraploid under high nutrient conditions ($1.08 \text{ g} \pm 0.086 \text{ g}$) had the lower aboveground biomasses than those growing alone ($1.56 \text{ g} \pm 0.072 \text{ g}$) or with a neotetraploid ($1.45 \text{ g} \pm 0.103 \text{ g}$), but plants growing with an established tetraploid did not differ from those growing with a diploid ($1.22 \text{ g} \pm 0.095 \text{ g}$). A competitor plant's ploidy level influenced belowground biomass accumulation for both diploids and established tetraploids under low nutrient conditions (Figure 2.1 C); however, under high nutrient conditions, a competitor's ploidy level influenced belowground biomass accumulation for diploids, established tetraploids, and neotetraploids (Figure 2.1 D.). Diploids grown with another diploid under low nutrient conditions were significantly lighter than those grown in any of the other competition regimes (Figure 2.1 C.), while established tetraploids grown with another established tetraploid were lighter than those grown with a diploid (Figure 2.1 C.) .Under high nutrient conditions, both established tetraploids and neotetraploids had the lightest belowground biomasses when grown with an established tetraploid, but this was only significantly different from all other competition regimes for focal established tetraploids (Figure 2.1 D.). Diploids grown with another diploid were significantly smaller than those grown alone under high nutrient conditions (Figure 2.1 D.).

Similarly, a plant's total biomass was influenced by focal plant ploidy, a competitor's plant ploidy, and the interaction among these factors, and the effects of these model factors depended upon soil nutrient supplies (Table 2.1 D.). Under low nutrient conditions, neotetraploids (Square root transformed LS Mean $\pm$ standard error; $1.04 \text{ g} \pm 0.181 \text{ g}$) were only significantly lighter than diploids ($1.68 \text{ g} \pm 0.157 \text{ g}$). Diploids ($1.80 \text{ g} \pm 0.113 \text{ g}$) were significantly lighter than established tetraploids ($2.38 \text{ g} \pm 0.105 \text{ g}$) under high nutrient conditions. In low nutrient conditions, plants growing alone ($1.55 \text{ g} \pm 0.128 \text{ g}$) or with a neotetraploid ($1.71 \text{ g} \pm 0.181 \text{ g}$) had the heaviest total biomass, while those growing with a diploid ($1.04 \text{ g} \pm 0.169 \text{ g}$) had the lowest, but none of the competition regimes differed from plants grown with an established tetraploid ($1.27 \text{ g} \pm 0.154 \text{ g}$). Plants growing with an established tetraploid under high nutrient conditions ($1.62 \text{ g} \pm 0.124 \text{ g}$) had a lower total biomass than those growing alone ($2.46 \text{ g} \pm 0.102 \text{ g}$) or with a neotetraploid ($2.28 \text{ g} \pm 0.148 \text{ g}$), but plants growing with an established tetraploid did not differ from those growing with a diploid ($1.88 \text{ g} \pm 0.136 \text{ g}$). A competitor plant's ploidy level influenced total biomass accumulation for both diploids and established tetraploids under low nutrient conditions; however, under high nutrient conditions, a competitor's ploidy level influenced total biomass accumulation for diploids, established tetraploids, and neotetraploids. Under low nutrient conditions, diploids grown with a diploid were the lightest. Established tetraploids grown with another established tetraploid were lighter than those grown with a diploid. Both established tetraploids and neotetraploids were lighter when grown with an established tetraploid under high nutrient conditions, and diploids grown with another diploid were also lighter under high nutrient conditions.

We did not detect any significant differences between the S:R ratio of plants based on focal plant ploidy, competitor plant ploidy, and their interaction, regardless of soil nutrient treatment (Table 2.1 E., Figure 2.1 E-F.).

Reproductive Traits- Out of our 325 surviving plants, only forty seven percent successfully flowered. Focal plant ploidy and the interaction among focal plant ploidy and competitor plant ploidy were found to influence a plant's likelihood to flower under both low and high nutrient supplies (Table 2.2). In both nutrient conditions, neopolyploids were the least likely to flower, while established polyploids were the most likely to flower in high nutrient conditions. Both diploids and established tetraploids were less likely to flower when growing with a plant of the same cytotype in either of the nutrient conditions. Competitor plant ploidy level did not affect neotetraploids' likelihood to flower under low nutrient conditions; however, under high nutrient conditions they were least likely to flower when grown with an established tetraploid. Of the plants that flowered, neither focal plant ploidy, competitor plant ploidy, nor their interaction influenced the number of flowers produced regardless of soil nutrient treatment (Table 2.3 A. Figure 2.2 A-B.).

We found that competitor plant ploidy influenced root bud production under both low and high nutrient levels, and that the interaction of focal plant ploidy and competitor plant ploidy was only significant under low nutrient levels (Table 2.3 B.). Under low nutrient conditions, plants grown alone (Square root transformed LS Mean $\pm$ standard error: 2.25 ± 0.170 root buds) or with a neopolyploid (2.37 ± 0.228 root buds) produced more root buds than those grown with a diploid (1.58 ± 0.215 root buds), but plants

grown with an established tetraploid (1.85 ± 0.198 root buds) did not differ in root bud production from either of these competitive regimes. Plants grown with a diploid (2.10 ± 0.152 root buds) or an established tetraploid (2.29 ± 0.139 root buds) under high nutrient conditions produced less root buds than those grown alone (2.89 ± 0.114 root buds); plants grown with a neotetraploid (2.44 ± 0.166 root buds) did not differ in root bud production. Only the root bud production of diploids was influenced by a competitor's ploidy level under low nutrient conditions; neither established tetraploids nor neotetraploids were influenced by their competitor under either nutrient level (Figure 2.2 C-D.) Diploids grown with another diploid produced the least amount of root buds under low nutrient conditions (Figure 2.2 C.).

Herbivory:

Only focal plant ploidy, but not nutrient level or the interaction among the two factors, affected the amount of leaf tissue consumed by *S. exigua* in the petri dish leaf choice bioassay (Table 2.4). Insects consumed the most from neopolyploids (Square root transformed LS Means $\pm$ standard error; $4.19\% \pm 0.004\%$), and the least from established tetraploids ($2.94\% \pm 0.326\%$). The amount of leaf area consumed from diploid leaves ($3.81\% \pm 0.319\%$) did not differ from the other two ploidy variations. A competitor's ploidy level was found to significantly impact *S. exigua* feeding damage from the whole plant caged bioassays, but only under high nutrient conditions (Table 2.5 A.). Insects consumed the most foliage from plants grown alone, and the least from plants growing with a diploid. Plants grown with established tetraploids and neotetraploids did not differ in the amount of damaged they received, nor did they differ from plants grown alone or

with a diploid. Furthermore, a plant's maternal genetic line and height at the time of the whole plant caged bioassay were also found to impact *S. exigua* feeding under low nutrient conditions. Insects consumed more foliage from taller plants and from certain genetic lines.

Focal plant ploidy, competitor plant ploidy, and plant height were all found to influence *F. occidentalis* feeding and were dependent upon soil nutrient conditions (Table 2.5 B.). The interaction of focal plant ploidy x competitor plant ploidy and maternal genetic line were found to be significant only under low nutrient conditions (Table 2.5 B.). Insects damaged more foliage from neotetraploids relative to both diploids and established tetraploids under both nutrient conditions. Under low nutrient conditions, plants grown alone experienced the least amount of *F. occidentalis* damage, while plants grown with an established tetraploids experienced the most. Plants grown with a diploid or a neotetraploid did not differ in the relative amount of damage they received, nor did they differ from plants grown alone or with an established tetraploid. The results of Tukey's HSD post-hoc test did not detect a significant difference among competitor plant ploidy levels under high nutrient conditions. Furthermore, under both nutrient conditions taller plants experienced more damage from *F. occidentalis*. Under low nutrient conditions, competitor ploidy level did not influence *F. occidentalis* damage patterns in diploids. However, established tetraploids grown with a diploid experienced more damage than those grown alone or with another established tetraploid, and neotetraploids grown alone experienced more damage than neotetraploids grown with an established tetraploid.

2.5 Discussion

Differences in ecological tolerances due to morphological and physiological distinctions between cytotypes is thought to influence how diploids and polyploids interact with their abiotic and biotic environments, subsequently affecting the competitive and consumption forces influencing the likelihood of neopolyploid establishment (Maherali et al. 2009, Manzaneda et al. 2012, Thompson et al. 2014, Thompson et al. 2015). However, not much is known about which abiotic factors are influential and how these factors may interact with different ploidy levels to influence competitive and herbivore-consumption forces differently between diploids and related polyploids (Thompson et al. 2015). Furthermore, even less is known about how these environmental factors influence neopolyploid performance relative to diploid performance. In this study, we tested the hypothesis that different nitrogen and phosphorus supplies would influence the competitive dynamics between diploid, established tetraploid, and neotetraploid cytotypes of *C. angustifolium*. We expected diploids, established tetraploids, and neotetraploids to differ in their performance traits due to the effects of genome duplication and/or evolution on the phenology of these cytotypes, and we expected the expression of these traits to change under different nutrient supplies when exposed to a competitor of a different ploidy level. For example, we had predicted that both established tetraploids and neotetraploids would exhibit suppressed performance when grown with a diploid under low nutrient conditions due to the differences in nutrient demands between cytotypes. Additionally, we tested whether nutrient supplies influenced insect feeding damage differently between diploids, established tetraploids, and

neotetraploids, and if a competing plant's cytotype also influenced foliar damage patterns. Overall, we found that whole genome duplication influences a plant's performance, and that these performance traits also depended on a competitor's cytotype and soil nutrient supplies. Furthermore, we found that herbivore foliar damage was also influenced by the interactive effects of soil nutrient supplies and whole genome duplication on a focal and competitor plant.

Soil nutrient supplies influences competitive dynamics:

Because polyploids require more nutrients relative to diploids to support their genome size (Leitch and Bennett 2004, Cavalier-Smith 2005, Guignard et al. 2017), we expected both neotetraploids and established tetraploids to be negatively affected by soil nutrient limitations. Therefore, we expected the competitive ability of polyploids to be partially dependent on soil nutrient availability. We found that diploids had larger belowground and total biomasses than neotetraploids under low nutrient conditions, but their biomasses did not differ from diploids grown in high nutrient conditions. The biomass of established tetraploids did not differ from diploids under low nutrient conditions; however, established tetraploids were significantly larger than diploids under high nutrient conditions. Additionally, we found that only established tetraploids and neotetraploids exhibited larger biomasses in the high nutrient conditions; diploid biomass did not significantly differ between nutrient treatments, which implies that polyploids are limited by nutrient scarcities, but diploids are not. Our findings agree with other recent studies that have found polyploids to perform better in high nutrient conditions than low nutrient conditions (Smarda et al. 2013, Guignard et al. 2016). This implies that soil

nutrient availability can influence the performance of diploids and polyploids differently, and that these changes in performance could impact a plant's competitive ability and subsequently the likelihood of a polyploid population establishing itself.

The cytotype of a competing plant was also found to differently influence the performance traits of diploids, established tetraploids, and neotetraploids under low and high nutrient supplies. Diploids and established tetraploids were most negatively influenced by a competitor when growing with the same cytotype under low and high nutrient conditions, respectively. Neotetraploids were not influenced by competition under low nutrient conditions, but under high nutrient conditions they performed poorly when growing with an established tetraploid. This is somewhat consistent with our previous findings (see Chapter 1) and with the findings of (Collins et al. 2011), where tetraploids were found to strongly compete with each other. However, it is interesting that the intensity of intra-cytotype competition within diploids and tetraploids varied based on soil nutrient availability, and that they were inverse of each other. This can be interpreted as evidence that both diploids and tetraploids can be strong competitors; however, the strength of their competitive abilities are dependent upon environmental factors. We did not see such a strong context-dependent interaction with neopolyploids. This could be because neopolyploids have not experienced the evolutionary adaption that defined the competitive traits of established polyploids.

We expected neopolyploids to be relatively weak competitors in comparison to established tetraploids because they have not been exposed to selection pressures selecting for strong performance traits. In line with our prediction, our findings suggest

that polyploidization does not instantaneous lead to a better competitor and that competitive ability may be an evolved trait in polyploids. Whole genome duplication is a large-scale mutation, and it can have severe consequences on mitotic and meiotic functioning and gene regulation leading to growth and fitness limitations (Soltis and Soltis 1999, Comai 2005, Cifuentes et al. 2010). Neotetraploids in our study had a significantly greater mortality rate relative to diploids and established tetraploids. The majority of neotetraploids died within the first five weeks of our experiment, and those that survived to the end of the experiment showed a great variance in performance traits such as height. For example, the shortest neotetraploid was 0.8 cm tall at the end of the experiment, while the tallest was 79.8 cm. Furthermore, the neotetraploids in our study were less likely to flower than diploids and established tetraploids. Neopolyploids often have lower fertility than related diploids due to higher frequencies of chromosomal irregularities during meiosis (Comai 2005, Cifuentes et al. 2010), which can result in nonfunctioning sexual structures and the failure to produce viable seeds (Soltis and Soltis 1999, Cifuentes et al. 2010). For example, one neotetraploid in our study exhibited a severely mutated flowering inflorescence (Appendix 3); while this plant was able to produce flowers, they were sterile. It is possible that high mortality rates and phenotypic variation present in our neotetraploids was also due to the multi-generational effects of using colchicine to induce polyploidy, as colchicine has been found to have lasting effects on plant morphology for several generations (Münzbergová 2017). Nevertheless, it is necessary for neopolyploids to overcome the natural disadvantages of polyploidization if they are to establish viable lineages. Therefore, early generation polyploids may face strong and immediate selection forces leading to the perpetuation of

the most fit genetic lines. Because our neotetraploid population was very young and had faced very few selection pressures until now, individuals likely to exhibited some of the negative effects of polyploidy. This observed bottleneck effect is one of the beginning steps to polyploid establishment, and one of the first selective forces shaping competitive ability. Furthermore, our findings demonstrate that variable soil nutrient supplies may be an abiotic factor influencing competitive dynamics in diploid-polyploid populations, and that relatively fertile soils may contribute to an ideal environmental condition better suited to facilitate to neopolyploid establishment.

Consumption dynamics influenced by whole genome duplication and nutrient supplies may affect competitive ability:

Herbivores tend to feed on healthy, vigorous plants (Cornelissen et al. 2008); however, traits contributing to the overall performance and fitness of a plant can be influenced by both biotic and abiotic factors (Houle 1997, Callaway et al. 2003, Wang et al. 2003, Shao et al. 2008). Because diploids and polyploids tend to have different morphological traits (Burton and Husband 2000, Otto and Whitton 2000, Segraves 2017), and because environmental factors are thought to differentially effect the performance of these traits (Thompson et al. 2014, Thompson et al. 2015), we predicted that herbivore damaging patterns would differ between diploids, established tetraploids, and neotetraploids under varying nutrient supplies and in the presence of competitors with different ploidy levels. We found that neotetraploids experienced more foliar insect damage relative to diploids and established tetraploids, regardless of soil nutrient treatment. This could imply that whole genome duplication alone does not influence traits

related to a plant's ability to resist herbivory, and that selective forces in a plant's natural habitat could be responsible for the observed differences in insect damaging patterns. However, because we looked at only two types of generalist feeding insects, we cannot make full conclusions as to the role of whole genome duplication in herbivore feeding preferences.

While we did not detect differences in insect cytotypic preference based on soil nutrient availability, it is possible that our nutrient treatments could have influenced the chemical composition of the cytotypes differently. For example, plants grown in nitrogen poor soils tend to exhibit a higher carbon:nitrogen (C:N) ratio, which is associated with greater concentrations of carbon-based secondary defense compounds due to the amounts of readily available carbon in the atmosphere (Bryant et al. 1983, Ibrahim et al. 2011). *Chamerion angustifolium* contains many carbon-based flavonoids (Maruška et al. 2014, Monschein et al. 2015), that are relevant to plant physiology as well as the way a plant interacts with its biotic environment (Mierziak et al. 2014). Flavonoids can also be used by plants as a form of chemical protection from insect pests (Simmonds and Stevenson 2001, Simmonds 2003, Mierziak et al. 2014). Because we did not measure the C:N ratio or concentrations of secondary defense compounds in our plants, we cannot conclude with full certainty that observed herbivore feeding patterns were not influenced by differential effects of the nutrient treatments on cytotypes. Pending data from analyses of the C:N ratio of our experimental plants will help determine if this ratio varied between cytotypes, if it was impacted by nutrient availability, and if it influenced observed herbivore feeding patterns.

We also found that competition regimes and the cytotype of a competitor plant influenced insect damage patterns. During the whole-plant cage bioassay, *S. exigua* consumed the most leaf area from plants grown alone in high nutrient conditions. We believe that this result was due to the number of larvae and plants present in each pot, as the two larvae placed in the “alone” competition treatment would have no choice but to feed from the same plant. This likely resulted in the greater amount of damage observed on plants grown alone. Additionally, some insects tend to feed more from nutritious plants grown in fertile soil (Waring and Cobb 1992, Butler et al. 2012), which may have also influenced *S. exigua* feeding patterns on plants growing alone. We also found that a competitor’s cytotype also influenced damage from *F. occidentalis*. Under low nutrient conditions, plants grown alone experienced the least amount of damage, while plants grown with an established tetraploid experienced the most. The small size and short life cycle of *F. occidentalis* may have influenced its feeding preferences. The *F. occidentalis* life cycle is typically complete after two weeks (Deligeorgidis et al. 2006), and we suspect that it may have been advantageous for these small insects to feed and oviposit on plants grown in competition due to the close proximity of two plants sharing a pot. However, we are not sure why *F. occidentalis* damaged the most foliage from plants grown with an established tetraploid, or why established tetraploids grown with a diploid experienced more damage relative to those grown alone or with another established tetraploid.

It is possible that other morphological and/or physiological differences between the cytotypes affected the feeding behavior of these insects. Studies evaluating cytotype-

herbivore interactions have found that the morphological and physiological differences between cytotypes can influence herbivore preference (Nuismer and Thompson 2001, Thompson et al. 2004, Halverson et al. 2008, Münzbergová et al. 2015), but the observed insect responses to ploidy level varies between different species of plants and insects (Bales 2015, Münzbergová et al. 2015). We are unaware of other studies comparing herbivore-feeding patterns between neopolyploids and other cytotypes nor are we aware of studies evaluating the effects of inter-cytotype and intra-cytotype competition on these feeding patterns. Therefore, it is difficult for us to determine how representative our results are to plant-insect feeding patterns involving neopolyploids.

Conclusion:

Neopolyploids face challenges establishing themselves as they are initially outnumbered in their diploid-dominant parental population (Levin 1975, Husband 2000). In order to overcome these challenges, neopolyploids must have a competitive advantage over diploids (Levin 1975, Fowler & Levin 1984, Husband 2000). Diploids and polyploids often display different morphological and physiological traits, as well as ecological tolerances; therefore, it is hypothesized that gradients of abiotic factors may influence competitive dynamics between the cytotypes influencing the likelihood of successful establishment (Thompson et al. 2015). Our results suggest that soil nutrient availability can influence competitive and consumption forces involving diploids and polyploids, potentially having a key role in the mechanisms leading to polyploid population establishment. Our study also suggests that neopolyploids are not competitively superior to diploids, but that their competitive ability can be influenced by

abiotic factors, such as soil nutrients, as we found competitive dynamics to change based on nutrient availability. Additionally, plants can experience tradeoffs in fitness and competitive ability through the physical damages and compensatory responses to herbivory (Borer et al. 2014), and soil nutrient availability can influence these responses (Lind et al. 2013, Borer et al. 2014,) through, for example, the formation of secondary defense compounds (Bryant et al. 1983, Ibrahim et al. 2011, De Long et al. 2015) and/or investment into rapid growth (Coley and Phyllis 1983). Therefore, plant-herbivore interactions can also be influenced by abiotic environmental factors, and they play a key role in shaping plant community structure (Eskelinen et al. 2012, Borer et al. 2014) and may also be instrumental in polyploid population establishment. Differences in competitive ability among cytotypes is held as a core mechanism to polyploid population establishment and our study shows that soil nutrients may influence these dynamics; yet, there is a lack in our understanding of the role relevant abiotic factors play in mediating competitive outcomes between cytotypes.

Recent anthropogenic changes in global environmental factors are expected to have large-scale, severe environmental and ecosystem consequences (Vitousek et al. 1997, Walther et al. 2002, Pecl et al. 2017), which may result in a change to abiotic factors relevant to cytotype competitive outcomes. For example, global nitrogen supplies are increasing due to agricultural practices and the over utilization of fossil fuels (Vitousek et al. 1997, Peñuelas et al. 2013, Fowler et al. 2015), resulting in major changes to plant community composition and diversity (Borer et al. 2014, Walter et al. 2017, Tatarko and Knops 2018). Our results suggest that soil nutrient availability may

have an important role in polyploid population establishment, and we may expect to see shifts in the prevalence and expansiveness of polyploid populations as global soil nutrients continue to increase. Additionally, many invasive species exhibit polyploidy, and in some cases only the polyploid variants are invasive (Pandit et al. 2011, te Beest et al. 2012, Rosche et al. 2017). By identifying biotic and abiotic factors contributing to polyploid establishment, we may better understand how these factors influence invasive species establishment and implement them into management and conservation plans, especially in the cases of polyploid invasive species.

We conclude that ideal environmental conditions may be necessary for polyploids to compete with their diploid progenitors and to successfully establish themselves. Our study suggests that soil nutrient availability, especially nitrogen and phosphorus, could be an important component to these ideal conditions. Studies focused on polyploid establishment have the potential to aid in our understanding of how plant community structure changes as a result of natural and anthropogenic changes, as well as how populations of invasive species establish themselves by overcoming similar barriers to neopolyploids.

2.6 Tables

Table 2.1: Results of a two-way, mixed-effects, full factorial ANOVAs with REML estimates testing focal plant ploidy (diploid, neotetraploid, established tetraploid), competitor plant ploidy (alone, diploid, neotetraploid, established tetraploid), their interactions on growth traits under low and high nitrogen treatments. Maternal genetic line was included in the models as a random effect. Degrees of freedom (k-1, N-k) are reported, and bold values indicate a significant *P*-value at $\alpha = 0.05$.

Factor	Source	Low Nutrients		High Nutrients	
		F _{df}	<i>P</i>	F _{df}	<i>P</i>
a. Final Height	Focal Ploidy (FP)	4.996 _(2,159)	0.0157	0.758 _(2,160)	0.4796
	Competitor Ploidy (CP)	1.977 _(3,158)	0.1200	4.140 _(3,159)	0.0075
	FP × CP	5.106 _(6,155)	<0.0001	5.733 _(6,156)	<0.0001
b. Aboveground Biomass (g)*	Focal Ploidy (FP)	3.354 _(2,159)	0.0528	9.553 _(2,156)	0.0011
	Competitor Ploidy (CP)	4.043 _(3,158)	0.0085	12.073 _(3,155)	<0.0001
	FP × CP	4.947 _(6,155)	0.0001	3.547 _(6,152)	0.0027
c. Belowground Biomass (g)*	Focal Ploidy (FP)	3.722 _(2,159)	0.0404	3.821 _(2,156)	0.0385
	Competitor Ploidy (CP)	2.850 _(3,158)	0.0396	7.811 _(3,155)	<0.0001
	FP × CP	4.933 _(6,155)	0.0001	3.151 _(6,152)	0.0063
d. Total Biomass (g)*	Focal Ploidy (FP)	3.556 _(2,159)	0.0451	7.389 _(2,156)	0.0036
	Competitor Ploidy (CP)	3.679 _(3,158)	0.0136	10.786 _(3,155)	<0.0001
	FP × CP	5.052 _(6,155)	<0.0001	3.455 _(6,152)	0.0032
e. Shoot:Root Ratio*	Focal Ploidy (FP)	1.039 _(2,159)	0.3986	3.069 _(2,156)	0.0690
	Competitor Ploidy (CP)	0.6490 _(3,158)	0.5850	0.790 _(3,155)	0.5016
	FP × CP	1.366 _(6,155)	0.2334	1.115 _(6,152)	0.3550

* Factor square root transformed

Table 2.2: Results of a two-way, fixed effects nominal logistic regression run under low and high nutrient treatments testing the effects of ploidy (diploid, established tetraploid, neotetraploid), competitor plant ploidy (alone, diploid, established tetraploid, neotetraploid), their interaction, and maternal genetic line on a plant's likelihood of flowering. Degrees of freedom (k-1, N-k) are reported, and bold values indicate a significant P -value at $\alpha = 0.05$.

Source	Low Nutrients		High Nutrients	
	X^2_{df}	P	X^2_{df}	P
Focal Ploidy (FP)	12.396 _(2,159)	0.0020	6.472 _(2,160)	0.0393
Competitor Ploidy (CP)	2.579 _(3,158)	0.4612	4.166 _(3,159)	0.2441
FP $\times$ CP	17.865 _(6,155)	0.0066	34.792 _(6,156)	<0.0001
Maternal Genetic Line	39.182 _(17,144)	0.0017	22.528 _(18,144)	0.2094

Table 2.3: Results of a two-way, mixed-effects, full factorial ANOVAs with REML estimates testing focal plant ploidy (diploid, neotetraploid, established tetraploid), competitor plant ploidy (alone, diploid, neotetraploid, established tetraploid), their interactions on reproductive traits under low and high nitrogen treatments. Maternal genetic line was included in the models as a random effect. Degrees of freedom (k-1, N-k) are reported, and bold values indicate a significant *P*-value at $\alpha=0.05$.

Factor	Source	Low Nutrients		High Nutrients	
		F _{df}	<i>P</i>	F _{df}	<i>P</i>
a. Number of Flowers*	Focal Ploidy (FP)	0.201 _(2,50)	0.8197	2.116 _(2,93)	0.1418
	Competitor Ploidy (CP)	0.368 _(3,49)	0.7767	1.717 _(3,92)	0.1699
	FP × CP	1.433 _(6,46)	0.2284	1.008 _(6,89)	0.4261
b. Number of Root Buds*	Focal Ploidy (FP)	2.391 _(2,159)	0.1156	3.255 _(2,155)	0.0735
	Competitor Ploidy (CP)	4.095 _(3,158)	0.0080	7.124 _(3,154)	0.0002
	FP × CP	2.335 _(6,155)	0.0351	1.468 _(6,151)	0.1937

* Factor square root transformed

Table 2.4: Results of the mixed-effects, full factorial ANOVAs with REML estimates testing focal plant ploidy (diploid, established tetraploid, neotetraploid), nutrient treatment (low, high), and their interaction on the percentage of leaf area consumed by *S. exigua* (square root transformed) during the petri dish bioassays. Maternal genetic line was included in the models as a random effect. Degrees of freedom (k-1, N-k) are reported, and bold values indicate a significant *P*-value at $\alpha = 0.05$.

Source	F _{df}	<i>P</i>
Focal Ploidy (FP)	3.770 _(2,237)	0.0477
Nitrogen (N)	0.0745 _(1,238)	0.7852
FP × N	0.3627 _(2,237)	0.6962

Table 2.5: Results of two-way, fixed effects ordinal logistic regression testing the effects of ploidy (diploid, established tetraploid, neotetraploid), competitor plant ploidy (alone, diploid, established tetraploid, neotetraploid), and maternal genetic line on insect feeding damage by *S. exigua* in the caged bioassays and by *F. occidentalis* at the end of the experiment under both low and high nutrient treatments. Plant height at the time of damage measurements was used as a covariate. Degrees of freedom (k-1, N-k) are reported, and bold values indicate a significant *P*-value at $\alpha = 0.05$.

Factor	Source	Low Nutrients		High Nutrients	
		X^2_{df}	<i>P</i>	X^2_{df}	<i>P</i>
a. Leaf Area Consumed in Cage Bioassay	Focal Ploidy (FP)	5.935 _(2,83)	0.0514	1.450 _(2,82)	0.4844
	Competitor Ploidy (CP)	1.465 _(3,82)	0.6903	14.007 _(3,81)	0.0029
	FP × CP	7.078 _(6,79)	0.3137	3.615 _(6,78)	0.7286
	Maternal Genetic Line	34.564 _(17,68)	0.0071	18.099 _(17,67)	0.3826
	Height (cm)	16.367 _(1,84)	<0.0001	0.097 _(1,83)	0.7558
b. Leaf Area Damaged by <i>F. occidentalis</i>	Focal Ploidy (FP)	11.881 _(2,158)	0.0026	14.730 _(2,160)	0.0006
	Competitor Ploidy (CP)	11.614 _(3,157)	0.0088	9.398 _(3,159)	0.0244
	FP × CP	16.059 _(6,154)	0.0134	9.875 _(6,156)	0.1300
	Maternal Genetic Line	27.842 _(17,143)	0.0468	21.416 _(18,144)	0.2589
	Height (cm)	29.642 _(1,159)	<0.0001	24.406 _(1,161)	<0.0001

2.7 Figures

Figure 2.1: The effects of the focal plant ploidy × competitor plant ploidy interaction under low and high nutrient conditions on (A-B) aboveground biomass (low: $F_{(6,155)}=4.947$, $P = 0.0001$; high: $F_{(6,152)}=3.547$, $P = 0.0027$), (C-D) belowground biomass (low: $F_{(6,155)}=5.052$, $P = 0.0001$; high: $F_{(6,152)}=3.151$, $P = 0.0063$), and (E-F) shoot:root

ratio (low: $F_{(6,155)}=1.366$, $P = 0.2334$; high: $F_{(6,152)}=1.115$, $P = 0.3550$). Square root transformed means $\pm$ SE. Different letters represent significantly different means within each focal ploidy grouping from Tukey's post-hoc HSD test.

Figure 2.2: The effects of the focal plant ploidy × competitor plant ploidy interaction under low and high nutrient conditions on (A–B) the number of flowers produce (low: $F_{(6,46)}=1.433$, $P = 0.22841$; high: $F_{(6,89)}=1.008$, $P = 0.4261$) and (C–D) the number of root buds produced (low: $F_{(6,155)}=2.335$, $P = 0.0351$; high: $F_{(6,151)}=1.468$, $P = 0.1937$). Square root transformed means $\pm$ SE. Different letters represent significantly different means within each focal ploidy grouping from Tukey’s post-hoc HSD test.

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Chapter 3: A summary of key findings

Through two greenhouse experiments, we tested the hypothesis that soil nutrient availability affects competitive outcomes among diploids and tetraploids. In the first experiment, we grew diploids and autotetraploids alone or in competitive regimes under low and high nitrogen treatments, while in the second experiment we grew diploids, established autotetraploids, and neotetraploids either alone or in competition under low and high nitrogen/phosphorus treatments. In both experiments, we looked at how competition and soil nutrient availability influenced performance traits and insect foliar damage on focal cytotypes. Overall, we found that genome duplication effects performance traits and plant-herbivore interactions, but these outcomes vary depending on nutrient supplies.

Focal cytotype and competitor cytotype influenced aboveground, belowground, and total biomass accumulation in both experiments, and these factors were dependent on nitrogen/nutrient treatments. However, the interaction among focal cytotype and competitor cytotype was found to be significant only in the second experiment, and it also was dependent upon nutrient treatment. In the first experiment, tetraploids were larger than diploids under low nitrogen conditions, while in the second experiment tetraploids were larger than diploids under high nutrient conditions. Tetraploids suppressed the growth of neighboring plants in the first experiment, regardless of soil nitrogen supply. However, in the second experiment, plants tended to be suppressed by diploids under low nutrient conditions, but under high nutrient conditions they tended to be suppressed by established tetraploids. Furthermore, in the second experiment we

generally found diploid growth traits to be suppressed by neighboring diploids under low nutrient conditions, and tetraploids to generally be suppressed by other tetraploids under high nutrient conditions. The shoot:root ratio was not found to be significantly influenced any model factors in either of our experiments.

Focal ploidy was only found to influence the likelihood of flowering in the second experiment, where under both nutrient conditions neotetraploids were least likely to flower. In the first experiment, only competitor plant ploidy influenced likelihood of flowering as under low nitrogen conditions plants grown with a tetraploid were least likely to flower, but under high nutrient conditions plants grown alone were more likely to flower. The interaction of focal ploidy and competitor ploidy was significant under both nutrient conditions in the second experiment, as both diploids and established tetraploids were least likely to flower when growing with a plant of the same cytotype regardless of soil nutrient treatment. In the first experiment, plants grown alone in high nitrogen conditions produced the most flowers. No other factors influenced flower production in either experiment. Furthermore, in the first experiment self- and cross-pollinated seed counts were not influenced by cytotype or nitrogen treatments. In the first experiment, diploids grown in high nitrogen conditions produced the most root buds; focal ploidy was not found to influence root bud production in the second experiment. In both experiments, the number of root buds produced by a plant was influenced by a competitor's ploidy under both nutrient conditions. Plants grown alone produced the most root buds in both experiments regardless of nitrogen treatment. In the second experiment, plants grown with a diploids under low nutrient conditions produced the least number of

root buds, and plants grown with a diploid or an established tetraploids produced the least number of root buds under high nutrient conditions.

Neither focal plant ploidy, competitor plant ploidy, nor their interactions under low and high nitrogen conditions influenced the foliar damage from *S. exigua* during the choice bioassays or the damage from *F. occidentalis* throughout our first experiment. However, in the second experiment, ploidy level and soil nutrient supplies influenced insect feeding damage. Both *S. exigua* and *F. occidentalis* consumed or damaged the most leaf area from neotetraploids relative to diploids and established tetraploids. During the whole plant choice bioassay, plants grown alone experienced the most damage from *S. exigua* under high nutrient conditions. Competitor plant ploidy also influenced *F. occidentalis* damage, as plants grown alone in low nutrient conditions experienced the least amount of damage, while plants grown with an established tetraploid under high nutrient conditions received the most. Furthermore, the interaction among focal plant ploidy and competitor plant ploidy influenced *F. occidentalis* feeding under low nutrient conditions, where diploids grown with diploids, and neotetraploids grown alone experienced more damage than neotetraploids grown with an established tetraploid.

Overall, the results of these experiments imply that the ploidy level of both a focal plant and its competitor are important factors influencing competitive outcomes, and that they can be affected by soil nutrient supplies.

Appendix

Appendix 1: Specific sample size for whole plant choice caged bioassays from chapter 2

Appendix 1: The number of pots per competition and nutrient treatment used in the whole-plant choice bioassays in chapter 2.

Competition Treatment	Nutrient Treatment	Number of Pots
Diploid (2x)- Alone	Low	12
	High	13
Established Tetraploid (4x) - Alone	Low	10
	High	11
Neotetraploid (4x') - Alone	Low	8
	High	7
2x v. 2x	Low	6
	High	4
2x v. 4x	Low	6
	High	6
2x v. 4x'	Low	3
	High	3
4x v. 4x	Low	6
	High	6
4x v. 4x'	Low	4
	High	4
4x' v. 4x'	Low	3
	High	3

Appendix 2: Results of three-way ANOVA from chapter 2

Appendix 2.1: Results of three-way, mixed-effects, full factorial ANOVA with REML estimates testing soil nutrients (low, high), focal ploidy (diploid, neotetraploid, tetraploid), competitor ploidy (alone, diploid, neotetraploid, tetraploid), and their interactions on growth traits. Degrees of freedom (k-1, N-k) are reported, and bold values indicate a significant P -value at $\alpha = 0.05$.

Factor	Source	F _{df}	P
a. Height (cm)	Nutrients (Nut)	33.179 _(1,323)	<0.0001
	Focal Ploidy (FP)	2.254 _(2,322)	0.1270
	Competitor Ploidy (CP)	5.271 _(3,321)	0.0015
	Nut × FP	6.676 _(2,322)	0.1270
	Nut × CP	0.450 _(3,321)	0.7176
	FP × CP	7.774 _(6,318)	<0.0001
	Nut × FP × CP	3.102 _(6,318)	0.0058
b. Aboveground Biomass (g)	Nutrients (Nut)	50.545 _(1,318)	<0.0001
	Focal Ploidy (FP)	4.420 _(2,317)	0.0227
	Competitor Ploidy (CP)	13.045 _(3,316)	<0.0001
	Nut × FP	8.664 _(2,317)	0.0002
	Nut × CP	1.830 _(3,316)	0.1418
	FP × CP	6.491 _(6,313)	<0.0001
	Nut × FP × CP	2.327 _(6,313)	0.0328
c. Belowground Biomass (g)	Nutrients (Nut)	40.618 _(1,314)	<0.0001
	Focal Ploidy (FP)	1.568 _(2,317)	0.2292
	Competitor Ploidy (CP)	8.556 _(3,316)	<0.0001
	Nut × FP	8.367 _(2,317)	0.0003
	Nut × CP	1.670 _(3,316)	0.1737
	FP × CP	6.362 _(6,313)	<0.0001
	Nut × FP × CP	2.074 _(6,313)	0.0562
d. Total Biomass (g)	Nutrients (Nut)	48.377 _(1,318)	<0.0001
	Focal Ploidy (FP)	3.157 _(2,317)	0.0599
	Competitor Ploidy (CP)	11.598 _(3,316)	<0.0001
	Nut × FP	8.595 _(2,317)	0.0002
	Nut × CP	1.873 _(3,316)	0.1343
	FP × CP	6.680 _(6,313)	<0.0001
	Nut × FP × CP	2.229 _(6,313)	0.0405
e. Shoot:Root Ratio	Nutrients (Nut)	1.603 _(1,318)	0.2066
	Focal Ploidy (FP)	0.263 _(2,317)	0.7779
	Competitor Ploidy (CP)	1.575 _(3,316)	0.1957
	Nut × FP	2.571 _(2,317)	0.0784
	Nut × CP	0.515 _(3,316)	0.6272
	FP × CP	1.085 _(6,313)	0.3719
	Nut × FP × CP	1.570 _(6,313)	0.1560

Appendix 2.2: Results of three-way, mixed-effects, full factorial ANOVA with REML estimates testing soil nutrients (low, high), focal ploidy (diploid, neotetraploid, tetraploid), competitor ploidy (alone, diploid, neotetraploid, tetraploid), and their interactions on reproductive traits. Degrees of freedom (k-1, N-k) are reported, and bold values indicate a significant *P*-value at $\alpha = 0.05$.

Factor	Source	F _{df}	<i>P</i>
b. Number of Flowers	Nutrients (Nut)	0.531 _(1,146)	0.4676
	Focal Ploidy (FP)	1.355 _(2,145)	0.2767
	Competitor Ploidy (CP)	0.586 _(3,144)	0.6253
	Nut × FP	0.485 _(2,145)	0.6170
	Nut × CP	1.062 _(3,144)	0.3678
	FP × CP	0.639 _(6,141)	0.6987
	Nut × FP × CP	2.081 _(6,141)	0.0603
a. Number of Root Buds	Nutrients (Nut)	16.944 _(1,318)	<0.0001
	Focal Ploidy (FP)	1.373 _(2,317)	0.2762
	Competitor Ploidy (CP)	10.291 _(3,316)	<0.0001
	Nut × FP	5.883 _(2,317)	0.0031
	Nut × CP	1.058 _(3,316)	0.3671
	FP × CP	1.613 _(6,313)	0.1433
	Nut × FP × CP	2.712 _(6,313)	0.0141

Appendix 3: Images of a mutant neopolyploid

Throughout the duration of our second experiment, we noticed a particular neopolyploid growing alone in a high nutrient treatment had begun showing strange inflorescent morphology. Here we documented the progression of its inflorescent development via photographs. While the plant was able to produce some normal-looking flowers that were sterile.

Appendix 3.1: Strange plant at approximately 12 weeks of growth. The stem is thin and flat, and the inflorescence is beginning to split in two!

Appendix 3.2: Strange plant at 16 weeks of growth. The stem is thickening near the flower buds as it splits into four small inflorescences.

Appendix 3.3: Strange plant at 17 weeks of growth. It flowers as the inflorescences continue to develop.

Appendix 3.4: Strange plant at 19 weeks of growth. The inflorescences continue to grow around each other, creating a knot!