Screening hybrid poplar clones for resistance to the forest tent caterpillar, *Malacosoma disstria*

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ABSTRACT

Screening hybrid poplar clones for resistance to the forest tent caterpillar, *Malacosoma disstria*

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Susceptibility of various hybrid poplar clones to attack by *Malacosoma disstria* larvae was assessed. No-choice experiments were conducted on one week old foliage using first instar larvae. Performance of larvae on each hybrid was also determined. Clones 3389 (DxB), 3729 (NxM), 505508 (MxDT), 750316 (MxT), and 915320 (MxB) were found to be highly resistant to attack by first instar larvae. Susceptible clones were found to belong to the *P. x euramericana* and *P. x generosa* Henry crosses. Hybrids with a *P. maximowiczii* or *P. balsamifera* parent were found to be consumed at intermediate levels. Consumption was found to be positively correlated to survivorship and negatively correlated to instar duration; instar duration was negatively correlated to survivorship.

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And last but not least, I would like to dedicate this thesis to my one and only love **William**, and our daughter **Rhea**.

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INTRODUCTION

The name *Populus* is derived from the Latin word *populi* which means "the people's tree" (Dickmann 2001). Currently, there is an ever-increasing demand for poplar wood supply (Berguson and Buchman 1998, Balatinecz and Kretschmann 2001), leading researchers to identify and propagate fast-growing plants. Some hybrid poplars display heterosis: they grow almost ten times faster than either parental species (Zalesny 2004), and can reach maturity within four years (U.S. Environmental Protection Agency. 1999).

In essence, a hybrid results from the fertilization of a flower of one species by the pollen of another. Screening is then conducted to select hybrids with desired traits of both parental species (Seitz 1958 as stated in Stanton et al. 2010). The highlight of the genus *Populus* is the ease with which they can be cloned (Dickmann 2001) via vegetative propagation. Stem cuttings of dormant sections of about 20-30 cm, otherwise known as sticks, can induce growth when planted (Dickmann 2001). *Populus tremuloides* is an exception to the rule as it is relatively difficult to clone from stem cuttings (Barry and Sachs 1968). It is this ability to be cloned effectively that gives poplars the potential for quick accumulation of favourable traits (DiFazio et al. 2011). Hybrid poplars clones have been screened in an attempt to select for those clones that display both resistance to pest attack and rapid growth (Robison and Raffa 1994). In general, screening of hybrid poplar clones is followed by clonal selection (selection of resistant trees for plantation or further crossing) (Ramirez et al. 2004). In my study, hybrid poplar clones were screened to isolate those clones that display resistance to, in particular, *Malacosoma disstria*.

Though hybrid poplars possess several valuable traits, they are also more susceptible to pest attack and have decreased reproductive fitness relative to either parental species (U.S. Environmental Protection Agency 1999, Broeck et al. 2005, Mattson et al. 2001). For instance, in the *Eucalyptus amygdalina x Eucalyptus risdonii* hybrid zone, there are almost 53% more species of insects and fungi per tree than in the pure parental zones and more generalists than specialists. The population of gall producing wasps is also almost 260 times more abundant in the hybrid zone relative to the pure zone (Whitham et al. 1994). In a study on poplar hybrids, Floate et al. (1993) noted that between 91.1 and 96.8% (over a three year period) of the *Chrysomela confluens* population was restricted to the hybrid zone and attributed the latter to early leaf flush of hybrids. Further, relative to the parental *Populus trichocarpa*, both the diploid and triploid hybrid showed a substantial reduction in pollen viability (a reduction from 98% in the parental type to 48% in diploids and 22% in triploids) and seed viability (a reduction from 92% in the parental type to 83-86% in diploids and 41-51% in triploids) (U.S. Environmental Protection Agency 1999).

Hybrids have also been shown to be less resistant to herbivores and pathogens than parental types. Orians et al. (2000) suggested that allocation of resources to growth results in lowered defense. Production of secondary compounds, the basis of resistance, is under genetic control such that hybrids, relative to parental genotypes, can show over-expression, under-expression, intermediate levels or levels similar to one or both parents. In particular, Orians et al. (2000) showed that levels of secondary compounds present in F1 willow hybrids are chemical intermediates between parental types (*Salix sericea, Salix eriocephala*). *S. eriocephala* has high levels of condensed tannins and no phenolic glycosides (salicortin, 2'-cinnamoylsalicortin) while *S. sericea* has ligh levels of salicortin and low levels of tannins and 2'-cinnamoylsalicortin. The

F1 hybrid possesses all three at intermediate levels, termed hybrid breakdown (the opposite of hybrid vigor) (Orians et al. 2000). Hybrid breakdown results from the splitting of co-adapted gene complexes as a result of recombination (Johansen-Morris and Latta 2006, Fritz et al. 1999). In one study of first-generation hybrids of the genus *Populus*, parental identity –chemical character expression or secondary compound expression of parental species- was retained 60% of the time, was lost 30% of the time and new co-adapted complexes were observed to form 10% of the time (Rieseberg and Ellstrand, 1993). Physical characteristics, such as trichome density, have also shown the intermediate effect in F1 *Alnus pubescens* hybrids (*Alnus glutinosa x Alnus incana*) (Grange 1995, Fritz et al. 1999).

Natural resistance to pests can result from phenological asynchrony, tolerance, growth compensation, lower nutritional quality and presence of defensive compounds (Clancy 2002). Asynchrony can lead to starvation and mortality of pests; early hatch results in starvation while late hatch presents the pest with an unsuitable source that decreases fecundity (Van Asch and Visser 2007). Jones and Despland (2006) showed that late-hatching forest tent caterpillars on aspen display slow growth, prolonged development, additional instars and a lower pupal mass.

Resistance can also be achieved through lowered foliage quality. In particular, the biomechanical properties and chemical constituents of leaves influence the growth of insect larvae. Chemical defense in poplars is based mainly on four compounds (salicin, salicortin, tremulacin, tremuloidin) that disrupt digestion by binding to proteins in the gut (Philippe and Bohlmann 2007). These compounds negatively impact performance of larvae (Hemming and Lindroth 1995, Philippe and Bohlmann 2007). In many trees of the temperate zone, leaf suitability to herbivores decreases over the growing season due to an increase in toughness, a decrease in

nutritional quality and an increase in defensive compounds. In fact, *Malacosoma americanum* caterpillars fed mature *Prunus serotina* (black cherry) leaves showed a decrease in growth that was correlated with low digestibility (Peterson 1987). Decrease in insect performance on mature foliage has been observed with both an increase in allelochemicals, (Hemming and Lindroth 1995) and an increase in leaf toughness (Raupp 1985, Hunter and Lechowicz 1992, Robison and Raffa 1994) Further, an increase in leaf age of has been associated with a 15%, 28% and 35% decrease in water, sugar, and nitrogen content in leaves of *Prunus serotina* (Schroeder 1986), and an increase in secondary metabolites in leaves of *P. tremuloides* (tannins, phenolic glycosides) (Jones and Despland 2006). *M. disstria* also performs better on young than on older *P. tremuloides* leaves, but later instar larvae are less affected by foliar age (Jones and Despland 2006).

Defoliation by pests, including *Malacosoma disstria*, decreases growth rates, wood production, quantity of stored food, and increases susceptibility of host to disease (Gregory and Wargo 1986), the extent of which is dependent on foliar age, location, time of defoliation and developmental stage of leaf. The fate of the defoliated host plant is dependent on the severity, frequency and timing of defoliation (Wargo 1978). In poplars, radial growth (Hildahl and Reeks 1960, Kosola et al., 2001, Reichenbacker et al., 1996, Bassman and Zwier 1993), height growth (Reichenbacker et al., 1996, Bassman and Zwier 1993, Tucker et al. 2004) and biomass (Reichenbacker et al., 1996, Bassman and Zwier 1993) of host were found to be affected by defoliation. Further, reductions in growth rates were found to be dependent on the extent of foliar damage (Tucker et al. 2004, Reichenbacker et al., 1996). Response and recovery of host is dependent on the extent of refoliation; foliar loss results in decreased respiration and growth causing a depletion of reserves, thereby leading to mortality (Wargo 1978). *P. tremuloides* and

Acer saccharum are capable of full foliar regeneration, however, most trees refoliate with smaller and fewer leaves (Gregory & Wargo 1986, Wargo 1978). This leads to a smaller photosynthetic area and thus less growth potential, production volume and energy storage (Wargo 1978). In fact, Wargo (1978) states that defoliated trees have less foliar material, twig and branch dieback, decreased terminal and radial growth, a retarded rate of wound closure and an impaired root system. Defoliation by insect herbivores can be a serious concern in tree plantations, and natural resistance to herbivores is therefore a valuable trait.

The genus *Populus* is divided in six sections: Abasa, Turanga, Leucoides (swamp poplars), Aigeiros (cottonwoods, black poplars), Tacamahaca (balsam poplars) and *Populus* (aspen, white poplars) (Dickmann 2001). Although inter-breeding is possible, there can be difficulties in crossing certain sections with others (intersectional crossing) primarily due to pre and post zygotic barriers or inviability of seed (Broeck et al. 2005). Pre-zygotic barriers include asynchronized flowering, pollen competition and incompatibility while post-zygotic barriers include hybrid inviability, breakdown and sterility (Broeck et al. 2005). In fact, it is always easier to cross species within the same section (intrasectional crossing). However, crosses between sections are carried out artificially (Gaget et al. 1989, Stettler et al. 1980). Amongst the 22-85 species belonging to the genus *Populus*, twelve are native to North America, of which: *P. deltoides, P. balsamifera, P. trichocarpa, P. grandidentata, and P. tremuloides* (Dickmann 2001, Broeck et al. 2005). *P. nigra,* and *P. maximowiczii* were introduced in North America as potential parental lineages for hybrids (Balatinecz and Kretschmann 2001, Eckenwalder 1996). The Direction de la recherche forestière du Ministère des Ressources Naturelles et de la Faune du Québec uses five main parents to generate hybrids: *Populus deltoides* (D), *P. balsamifera* (B), *P. maximowiczii* (M), *P. trichocarpa* (T), and *P. nigra* (N) (Perinet 2007). Hybrid or clonal selection

is based on traits like growth, cold hardiness, tree form, disease and insect resistance, site adaptability, and wood quality (Perinet 2007). Hybrids of *Populus deltoides* and *Populus trichocarpa* display high heterosis (Marron et al. 2007), and low resistance (Ramirez et al. 2004), Further, hybrids of *Populus nigra* also display low resistance (Ramirez et al. 2004). Hybrids of *Populus maximowiczii* are adapted to colder conditions and forest sites (Perinet 2007). In particular, *Populus maximowiczii* crossed with either *P. balsamifera* or *P. trichocarpa* show superior growth and adaptability to forest soils (Perinet 2007) and high resistance to pest attack (Ramirez et al. 2004).

Of the 300 species of insects and mites in North America and the 525 species in Europe that damage trees belonging to the genus *Populus*, coleopterans and lepidopterans are the main defoliators (Mattson et al. 2002). In particular, *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae), the forest tent caterpillar, is a common leaf-chewing outbreak defoliator species of deciduous trees. *Malacosoma disstria* larvae are dark with white spots and blue lines on the dorsal side. Egg masses, composed of 100-350 eggs, hatch in synchrony with the bud break (emergence of leaves) of the host tree after a period of diapause by using photoperiod and temperature cues (Van Asch and Visser 2007). *M. disstria* is a temperature-dependent synchronized nomadic forager (Peters and Despland 2006, Schultz 1983) whose group movements are accompanied by semiochemical communication (a silk-pheromone trail) permitting cohesion of colony, increasing foraging efficiency and likelihood of survival (Fitzgerald 1995). Group living permits thermoregulation, increases foraging potential and anti-predatorial defence; however, it also leads to diminished growth, resource partitioning, competition and increased likelihood of pathogen or disease transmission (Fitzgerald 1995). This nomadic caterpillar species does not build a silk shelter (Fitzgerald 1995). Rather, as foraging

progresses, silk mats are woven, rested upon and abandoned (Fitzgerald 1995). However, fourth instar caterpillars adopt a solitary lifestyle – an ontogenetic shift (Fitzgerald 1995, Despland and Hamzeh 2004). The larvae undergo five to six molts before pupating and emerging as a moth. The moth does not feed; after eclosion, it mates, lays eggs and dies. And the cycle begins anew (Fitzgerald 1995).

Food choice by caterpillars depends on a variety of source traits, including sugars, secondary metabolites (Meyer and Montgomery 1987, Hemming and Lindroth 1995), leaf morphology (Rivero-Lynch et al. 1996), foliar moisture (Robison and Raffa 1994), leaf toughness (Hunter and Lechowicz 1992) & foliage availability. Excitatory and inhibitory neural signaling transmitted from insect taste receptor systems as a result of phagostimulants and deterrents in source dictates larval behaviour and host acceptability with host rejection associated with deterrent levels (Chapman 2003, Albert and Parisella 1985).

Malacosoma disstria uses trembling aspen (*Populus tremuloides*) as one of its most common host plants. Time to pupation and pupal weight of *Malacosoma disstria* larvae were found to be lower and higher, respectively, on *Populus tremuloides* than on *Acer saccharum*, another host plant (Lorenzetti et al. 1999). This difference in host quality is thought to be linked to the difference in phenolics concentration: eight times higher in *Acer saccharum* than in *Populus tremuloides* (Lorenzetti et al. 1999).

This thesis examines the susceptibility of hybrid poplars (*Populus* sp) to attack by the forest tent caterpillar (*Malacosoma disstria Hübner*). Previous studies conducted on resistance of hybrid poplar clones to second and fourth instar larvae showed that *Malacosoma disstria* larvae can

discriminate amongst hybrid poplar clones, especially early instar larvae that are highly selective (Robison and Raffa 1994). In my study, leaf consumption by first instar larvae was used to rank hybrids. I also compared time to reach the second instar and survival to the second instar between hybrid poplar clones. It is hypothesized that larvae on a preferred clone (i.e. those that show a high consumption rate) will show high levels of survival and rapid development. The purpose of this study is to rank hybrid poplar clones in terms of their quality as hosts for first instar larvae of *M. disstria*.

MATERIALS AND METHODS

POPLAR HYBRIDS, YEAR 2010 & 2011

Sixteen clones were obtained from Dr. F. Lorenzetti, representing crosses between 5 parental species (Table 2.1). In May of 2009, saplings representing some of the clones were brought from Ripon, Quebec to the UQAM greenhouse. One month later, cuttings were received for the remaining clones (for a total of 16 clones) which were induced to grow in 2:1 sand: peat moss soil mixture. All saplings were allowed to overwinter outside and were subsequently brought back into the greenhouse in spring 2010. Furthermore, they were treated with fertilizers, insecticides and fungicides when necessary to prevent damage by pests and to promote growth (Ralph 2012). Experiments were carried out between April and June 2010; clones 916401, 915508, 915319, 915302, 3729, 3565, 3333, 3230, and 3225 were used with one week old foliage and first instar caterpillars.

In 2011, a new set of 30 saplings were received from Ripon (Quebec) in December 2010. They were potted, covered and left to overwinter outside at Concordia University. These plants were

not treated with insecticides, pesticides or any other chemical products; they were left to grow under natural conditions. Testing of 15 clones – 131, 3225, 3230, 3308, 3333, 3374, 3389, 3565, 3729, 505508, 750316, 915313, 915319, 915320 & 915508 - took place between May and July 2011 on 1st instar caterpillars using one week old leaves.

FOLIAGE OF *Populus tremuloides*, YEAR 2010 AND 2011

In 2010, foliage required for the rearing of FTC larvae was collected at either Angrignon Park (located in Montreal, Qc, Canada) or Saint-Esprit (coordinates: N 45 55 21 7, W 73 39 55 7). Large branches were collected, brought to the lab and placed in tap water.

In 2011, *Populus tremuloides* foliage was collected at the Parc Nature du Bois-de-Liesse (Pierrefonds, Québec). The branch was cut and was placed in a water filled jar until needed for either rearing the caterpillars or for experimental use.

REARING: EGG MASS - 1ST INSTAR, YEAR 2010

Egg masses were collected at Saint-Esprit on April 8th, 2010. They were stored at 4°C until needed at UQAM. Egg masses were cleaned for 1 minute in a 6% sodium hypochlorite solution to destroy any viral pathogens that may have been present and were then rinsed in tap water for another minute. The egg masses were placed in a small plastic container lined with wet paper towel and wax paper. The experimental set-up was then placed under a UV lamp with a 16:8 L:D photoperiod; caterpillar hatch was monitored daily. Once hatched, they were used in no-choice experiments. Experiments were carried out from April to June of 2010.

In 2011, egg masses were collected from various sources and used indiscriminately:

(i) *Owen Sound, near Barrie, Ontario in the latter part of February (supplied by Dr. Lorenzetti)*

(ii) *Ontario, collected in early spring 2011 by the Canadian Forest Service*

(iii) *British Columbia, collected by Steffan Lindgren*

Rearing was conducted at Concordia under the same conditions as in 2010.

EXPERIMENTAL SET-UP, YEAR 2010

Leaves were used in experiments one week after budbreak. A single leaf (a hybrid leaf or a *Populus tremuloides* leaf) was placed in a water-filled eppendorf tube. The latter was then placed in a Petri dish lined with moist paper towel and wax paper. Ten hatchlings were then placed on top of the leaf and allowed to feed.

The experiment lasted for 48 hours. Leaves, ***after consumption***, were scanned and quantity eaten was assessed using ImageJ (scanned images of leaves).

A group of ten caterpillars were used per experiment, which was repeated between six to ten times for each of the 8 clones tested in the NC (No-Choice) experiment. *P. tremuloides* was used as a control.

EXPERIMENTAL SET-UP, YEAR 2011

The same set-up was used once again in 2010, except that the eppendorf was filled with cotton to ensure that the stem of the leaf remained in water at all times, thereby slowing or preventing water loss and preventing air bubbles near the petiole insert. Initial and final photographs of the leaf were used to calculate consumption during the 48 h experimental period. Either eleven or twelve replicates were conducted for each of the 15 clones tested. *P. tremuloides* was used as a control (replicated 12 times). In each case, the experimental set-up for each hybrid was accompanied by a negative control (replicated 5 times) which consisted of a single leaf without any caterpillars.

After 48 hours, the leaf was taken out and replaced with a fresh leaf. The experiment was continued until the whole group moulted to the second instar, in order to record growth and survival. Leaves were replaced as necessary.

METHODOLOGICAL APPROACHES OF LEAF AREA CONSUMED

There are two possible ways of calculating surface area consumed:

(i) Holes Method, which involves calculating the amount consumed by measuring the area of the holes in the final image (done in ImageJ by tracing the contour of the hole). The problem associated with this technique is that consumption often starts at the edge of a leaf and therefore the size of the "hole" is difficult to estimate.

(ii) Difference Method, which involves taking the difference between the initial and the final leaf area.

In 2010, after 48 hours, leaves used in the no-choice experiments were taken, cleaned and taped onto a piece of paper. Leaf surface area consumed was then calculated by measuring the "holes" in the leaf using scanned images and ImageJ. This however was problematic when the amount of leaf consumed was large, as it was difficult to estimate the original leaf contour without initial images. Leaves for which it was impossible to reliably estimate consumption were omitted from the analyses.

In 2011, a different approach was used; both initial and final pictures (after 48 hours) of leaves were taken in order to use the difference method. However, a different set of problems were encountered.

Leaf surface area of control leaves (without larvae) was found in many cases to be either larger or smaller after 48 hours, suggesting that the leaves either grew or shrank during the experiment. Therefore, most images were analyzed again using the holes technique to avoid this particular problem. The difference method was only used when most or all of the leaf was consumed.

In most cases, the holes method was the most frequently used method to calculate area consumed. In 2011, when it was impossible to measure area using the holes method (when leaf fully consumed for instance), the difference method was used instead.

STATISTICAL ANALYSIS

Data was analyzed using SPSS (version 16.0). Surface area consumed was compared between hybrids using a non-parametric test, the Kruskal-Wallis test; the data set did not pass tests of normality or homoscedasticity. Further, Spearman correlations were carried out between instar duration, survivorship and surface area consumed.

RESULTS

SURFACE AREA CONSUMED (48 HOURS), YEAR 2010

All leaves were consumed to some extent, with the highest consumption found in *P. tremuloides*. In this case, only the hole method (refer to methods; problems in methodology) was used to assess consumption. Consumption was found to differ between tested plants (8 clones – 3225, 3230, 3333, 3565, 3729, 915319, 915508, & 916401; Kruskal-Wallis, X^2=27.488, df=8, p=0.001). Both clones 915508 (DNxM) and 3565 (DxN) were found to be in the upper half of the scale (highly consumed). Crosses that included *P. maximowiczii (3729- P. nigra x P. maximowiczii and 915 302- P. maximowiczii x P. balsamífera)* were in the lower half (Figure 1, Table 2, Table 4).

SURFACE AREA CONSUMED (48 HOURS), YEAR 2011

In 2011, 15 clones – 131, 3225, 3230, 3308, 3333, 3374, 3389, 3565, 3729, 505508, 750316, 915313, 915319 & 915320 - were tested, each replicated 12 times (with the exception of clone 3729 for which N=11). In this instance, both the hole and difference methods were used to

calculate area consumed. Leaf surface area consumed was found to differ between plants (clones & *P. tremuloides*). Leaf surface area consumption of *P. tremuloides* was found to be greater than that of any clone (mean: 1.89 ± 0.21 cm^2). Clone 3389 (DxB) showed the lowest consumption (0.06 ± 0.04 cm^2). Several clones show similar low consumption values: 3729 (NxM; 0.28 ± 0.06 cm^2), 915320 (MxB; 0.29 ± 0.05 cm^2), 750316 (MxT; 0.28 ± 0.05 cm^2), and 505508 (MxDT; 0.28 ± 0.06 cm^2). Further, surface area consumed was negatively correlated to instar duration and positively correlated to survivorship (Figure 2, Table 2, Table 4).

SURFACE AREA MEASUREMENT ERROR OF CONTROL LEAVES, YEAR 2011

Change in leaf surface area of control leaves, measured with the difference method, showed an increase in the final **control** leaf surface area for many of the 15 clones (n=5). Error ([initial area-final area]/initial area) was found to be different between hybrids. In particular, the negative control leaves of clone 3729 were found to have decreased by 39.2% in a 48 hour period while that of clone 3565 showed a decrease of 41.6% (with a large standard error) during the same time frame (Figure 3, Table 2).

INSTAR DURATION, YEAR 2011

The number of days required to molt to the second instar was recorded for 14 clones – 3225, 3230, 3308, 3333, 3374, 3389, 3565, 3729, 505508, 750316, 915313, 915319 & 915320 – as well as *P. tremuloides* (N=12 in each with the exception of clone 750316 for which N=11). The number of days required to molt was found to differ between plants (clones and *P. tremuloides*). The average number of days to molt for *P. tremuloides* was 5.5 ± 0.29 (days), which was lower than that of the hybrids (Table 2, Table 3, Table 4).

SURVIVORSHIP, YEAR 2011

Survivorship was recorded on 14 clones – 3225, 3230, 3308, 3333, 3374, 3389, 3565, 3729, 505508, 750316, 915313, 915319 & 915320 – as well as *P. tremuloides* (N=12 in each with the exception of clone 750316 for which N=11). Survivorship (the number of caterpillars that survived to molt to the second instar) was found to differ on the various plants. Survival was highest on clones 3308 (DxN), 3565 (DxN) and *P. tremuloides* and lowest on 750316 (MxT), 505508 (MxDT), and 3389 (DxB). Further, a negative significant relationship was observed between instar duration and survivorship (Table 2, Table 3, Table 4).

DISCUSSION

Of the eight clones tested in 2010 (one week old foliage), clone 3729 (NxM) was the least consumed by larvae. Robison and Raffa (1994) found that NM6 (NxM, though not the same clone) was fast growing, and both defoliation tolerant and resistant to attack by the forest tent caterpillar. In 2011, five clones – 3389 (DxB), 3729 (NxM), 505508 (MxDT), 750316 (MxT) and 915320 (MxB) - were consumed at low levels by first instar larvae. Leaf surface area consumed on one week old foliage was highest on *P. tremuloides* and lowest on clone 3389 (DxB). Clone 3389, the least suitable host, is a cross between *P. deltoides* and *P. balsamifera*. Rejection of foliage is possibly due to the fact that *P. balsamifera* is fairly resistant to insect damage (mostly by the forest tent caterpillar and the Poplar borer) due to the presence of terpenes and resin covered buds (Zasada and Phipps 1990). *P. deltoides,* however, does not display this resistance. It is however fast-growing (Dickmann 2001). The *P. deltoides* clone tested by Robison and Raffa (1994) was found to be a suitable host for *M. disstria;* larvae on clone NC11004 (*P. deltoides*)

grew approximately 2 to 3 times faster than those on NC11505 (MxT) and displayed a high survival rate. In my experiment, all clones in 2011 isolated as resistant to *M. disstria* possess *P. maximowiczii* as a parent, with the exception of clone 3389 (DxB). This trend is similar to one noted in a study by Ramírez et al. (2004) which showed that density of *Chaitophorus leucomelas* (Homoptera: Aphididae) was found to be lowest on hybrids with a *P. maximowiczii* parent. Ramírez et al. (2004) attributes this parental resistance to the tough lower leaf surface of *P. maximowiczii* parents. *P. deltoides* and *P. trichocarpa*, on the other hand, were found to harbor large aphid densities (Ramírez et al. 2004).

The interamerican (*P. deltoides x P. trichocarpa, P. trichocarpa x P. deltoides*) and the euramerican (*P. deltoides x P. nigra*) hybrids are known to exhibit fast growth and to be highly productive. In my 2011 study, clones 131 (*P. deltoides x P.nigra*), 3225 (*P. trichocarpa x P. deltoides*), 3230 (*P. trichocarpa x P. deltoides*), 3308 (*P. deltoides x P. nigra*), 3333 (*P. deltoides x P. nigra*), 3565 (*P. deltoides x P. nigra*) and 915319 (*P. maximowiczii x P. balsamifera*) were found to be the most susceptible to larval attack. With the exception of clone 915319, these susceptible clones all belong to the interamerican or euramerican category. In contrast, data collected in 2010 shows clones 3225 (TxD), 3230 (TxD) and 3333 (DxN) as fairly resistant (medium resistance). This difference (between years) could be due to the *lower* number of replicates, the technique used to compute area (in 2010) or due to the environmental conditions to which the clones were subject. Due to the problems in methodology in 2010, whereby leaf area consumed was computed by measuring the holes in the leaf, and the low number of replicates, data collected in 2010 was subject to more variation, and thus error. In addition to what was observed in this study, another study on hybrid poplar clones using late-instar larvae of

M. disstria shows a similar trend. A study by Ralph (2012) showed that clones 3333 (DxN), 3308 (DxN), 3225 (TxD), 3230 (TxD) and 131 (DxN) all belonged to the intermediate or high preference group in experiments conducted with older larvae. Ralph (2012) also showed that clones 3729 (NxM), 3374 (BxM), 915302 (MxB) and 915313 (MxB) are poorly consumed. Ralph (2012) observed that hybrids with a *P. balsamifera* parent displayed high resistance to *Malacosoma* larvae; while those with a *P. maximowiczii* or *P. deltoides* parent displayed intermediate resistance and those with a *P. euramericana* parent displayed low resistance to Malacosoma larvae. In my study, low resistance was found to be centered around the *P. x euramericana* and *P. x generosa* Henry crosses. Hybrids with a *P. maximowiczii* or a *P. balsamifera* parent were found to display intermediate resistance – found in all three categories (high, medium, and low). However, neither I nor Ralph (2012) have observed *P. x euramericana* (DxN) or *P. x generosa* (TxD) crosses in the highly resistant category. Assuming Ralph's (2012) ranking as the preference model, and comparing Ralph's (2012) ranking to the one I generated, one can note a few discrepancies. In the first case, my data was not found to be consistent over the two year study period. In the second case, were we to recommend parents, I would recommend against the *P. deltoides* parent - recommended by Ralph (2012). This difference could either be due to our method of ranking (different set of hybrids, and different method of ranking) or to larval age (Ralph (2012) dealt with fourth/fifth instar larvae while I dealt with first instar larvae).

The number of days to molt on young foliage was found to be lowest on *P. tremuloides*, and on clones 3308 (DxN) & 3565 (DxN), both of which are crosses between *P. deltoides* and *P. nigra*. On clones 3389 (DxB), 505508 (MxDT), 750 316 (MxT), 915 320 (MxB) and 3374 (BxM), the development time was almost twice as that on *P. tremuloides*. Further, in this study, a negative

correlation was found between instar duration and survivorship and between surface area consumed and instar duration. On the other hand, a positive correlation was found between surface area consumed and survivorship. This suggests that a number of clones show a consistent effect on *M. disstria* performance*:* low consumption, longer development time and low survival. A study by Robison and Raffa (1994) found that there exists a negative correlation between survival and development time (R= -0.77) of *Malacosoma disstria* larvae on different poplar (clone) hosts. On the other hand, L2 preference was found to be positively correlated to survival and negatively correlated to development time (Robison and Raffa 1994).

High first instar caterpillar mortality rates are often observed with lepidopteran larvae (Zalucki et al. 2002). Shiga (1979) (as stated in Fitzgerald 1995) noted a 29.8% mortality by the end of the second stadium in *Malacosoma neustrium* while Filip & Dirzo (1985) (as stated in Fitzgerald 1995) noted a 39.7% mortality in *M. incurvum*. In this particular experiment, most of the hybrids as well as *P. tremuloides* were found to have a first instar caterpillar mortality rate between 4-17.5% on one week old foliage; however, clones 3729, 3389, 750316 and 505508 were found to have a mortality rate of 38.3%, 61.7%, 40% and 37.5% respectively by the end of the first instar.

In essence, susceptibility to attack by *Malacosoma disstria* was found to be lowest on clone 505508 (MxDT), 750316 (MxT), 3729 (NxM), 915320 (MxB), and 3389 (DxB). Further, crosses belonging to the *P. x euramericana* and *P. x generosa* category were found to be quite susceptible to attack by *Malacosoma disstria*.

Table 1: List of hybrid poplars received and tests conducted in 2010 and 2011 (Where D=*Populus deltoides*; N=*Populus nigra*; B=*Populus balsamifera*; M=*Populus maximowiczii*; T=*Populus trichocarpa*).

Clone Number	Cross	Tested in 2010	Tested in 2011
131	D x N		Figure 2 Figure 3
3225	T x D	Figure 1	Figure 2 Figure 3 Table 3
3230	T x D	Figure 1	Figure 2 Figure 3 Table 3
3308	D x N		Figure 2 Figure 3 Table 3
3333	D x N	Figure 1	Figure 2 Figure 3 Table 3
3374	B x M		Figure 2 Figure 3 Table 3
3375	B x M		
3389	D x B		Figure 2 Figure 3 Table 3
3565	D x N	Figure 1	Figure 2 Figure 3 Table 3
3567	D x N		
3570	D x N		
3585	D x N		
3586	D x N		
3587	D x N		
3729	N x M	Figure 1	Figure 2 Table 3
4813	D x N		
505299	M x DT		
505372	M x DT		
505508	M x DT		Figure 2 Figure 3 Table 3

747210	B x T		
750301	M x T		
750316	M x T		Figure 2 Figure 3 Table 3
915004	B x M		
915005	B x M		
915302	M x B		
915303	M x B		
915308	M x B ???		
915311	M x B		Figure 3
915313	M x B		Figure 2 Figure 3 Table 3
915318	M x B		
915319	M x B		Figure 2 Figure 3 Table 3
915320	M x B		Figure 2 Figure 3 Table 3
915508	DN x M	Figure 1	Figure 2 Table 3
916401	DN x M	Figure 1	Figure 3

Note: List received from Dr. Lorenzetti (Email: francois.lorenzetti@uqo.ca; Contact information: Université du Québec en Outaouais, C-3312, Pavillon, Ripon, Canada)

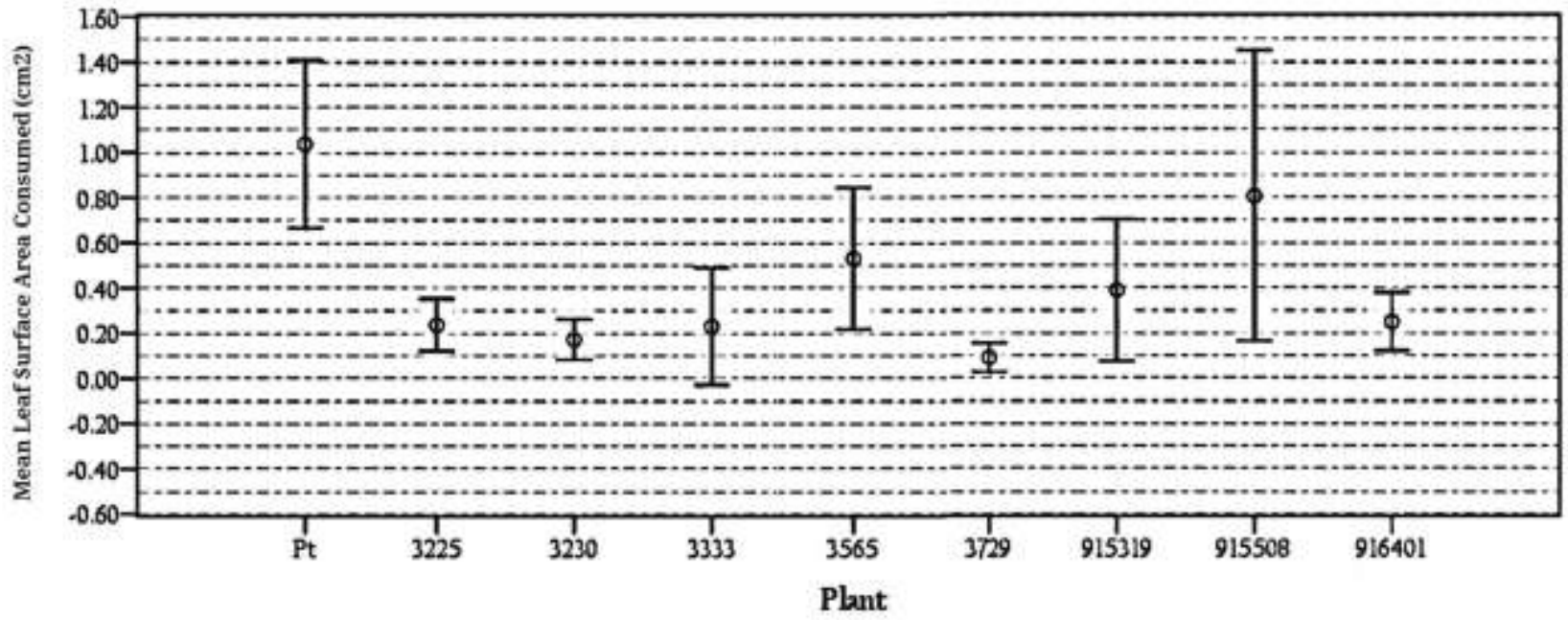

Figure 1: Mean Surface Area Consumed (± 2 S. E.) in No-Choice experiments conducted in 2010 using the holes method (One week old foliage; N=6 for all data used in the experiment except for *P. tremuloides* (N=8), 3225 (N=7), 3729 (N=9) and 916401 (N=8))

Table 2: Statistical tests performed for experiments conducted in 2010 and 2011 (Where p=Probability, DF = degrees of freedom).

Year	Experiment	Test	Statistic	p	DF	N
2010	Surface Area Consumed (SAC)	Kruskal-Wallis	$\chi^2 = 27.488$	p=0.001	Df = 8	—
2011	Surface Area Consumed (SAC)	Kruskal-Wallis	$\chi^2 = 114.685$	p<0.0005	Df = 15	—
2011	Surface Area Measurement: Error of Control Leaves	Kruskal-Wallis	$\chi^2 = 61.131$	p<0.0005	Df = 16	—
2011	Instar Duration	Kruskal-Wallis	$\chi^2 = 110.999$	p<0.0005	Df = 14	—
2011	Survivorship	Kruskal-Wallis	$\chi^2 = 69.432$	p<0.0005	Df = 14	—
2011	Instar Duration versus Survivorship	Spearman Correlation *(For clones in Table 3)*	ρ= -0.570	p<0.0005	—	N = 179
2011	SAC versus Instar Duration	Spearman Correlation *(For clones in Table 3)*	ρ= -0.706	p<0.0005	—	N = 178
2011	SAC versus Survivorship	Spearman Correlation *(For clones in Table 3)*	ρ= 0.614	p<0.0005	—	N = 179

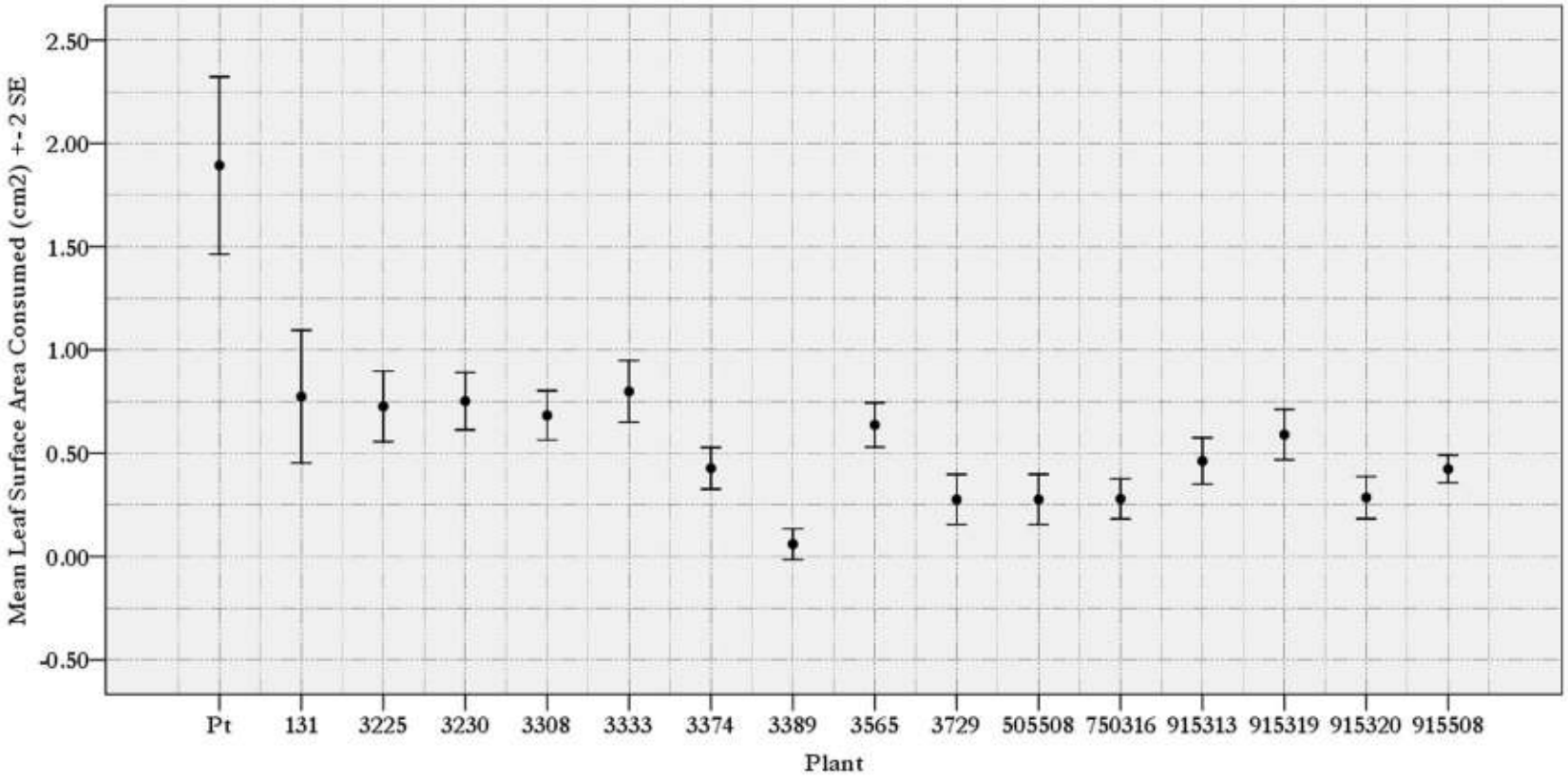

Figure 2: Mean Surface Area Consumed (± 2 S. E.) in the 2011 experiment at 48 hours using a combination of the hole and the difference method. (N=11 for clone 3729; N= 12 for the remaining clones)

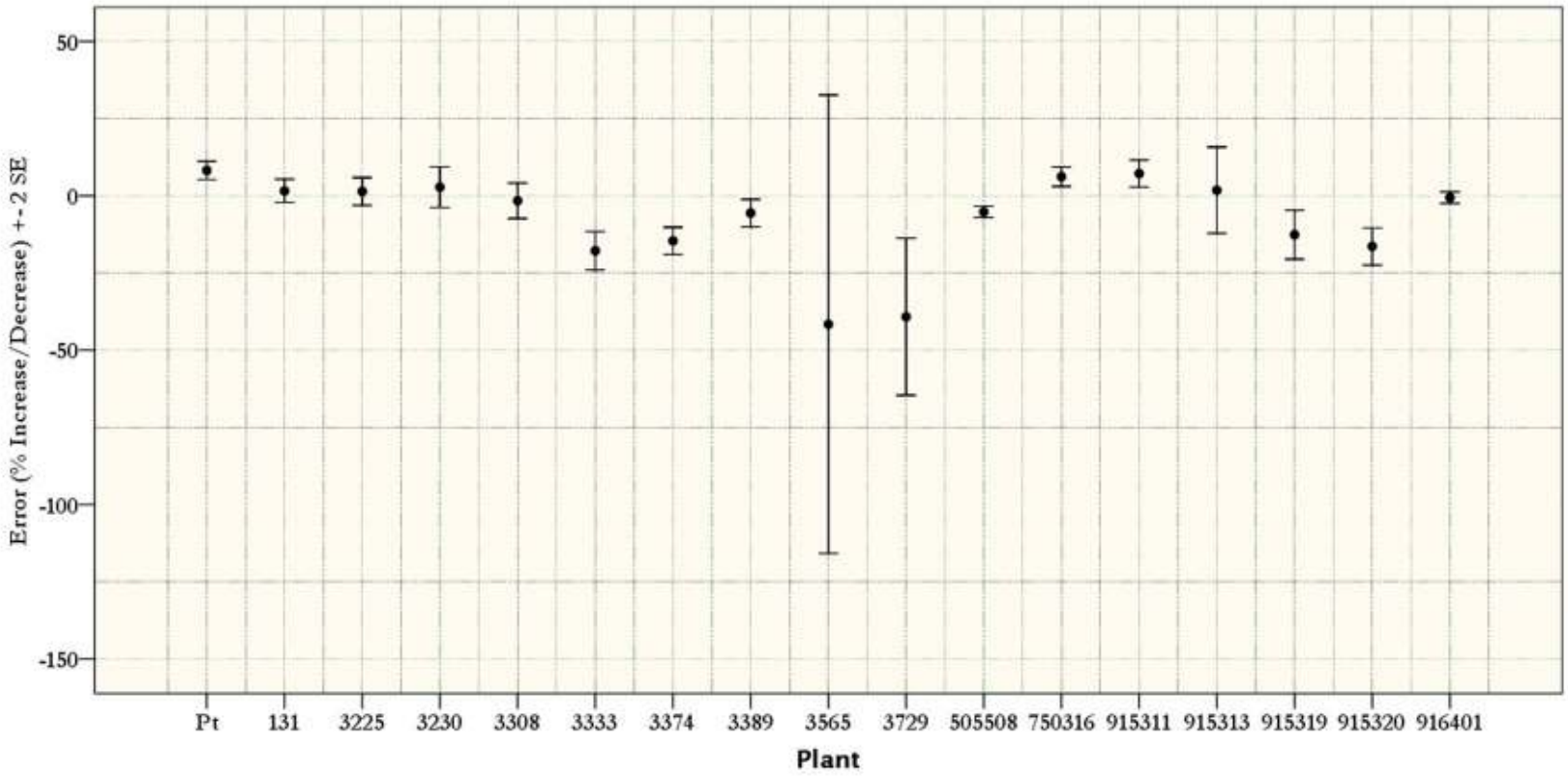

Figure 3: Error (or % increase or decrease ± 2 S. E.) of Negative Control leaves (*P. tremuloides* leaves without larvae) using ImageJ in the 2011 No-Choice experiment at 48 hours. (Where error = [initial area – final area]/initial area; N=5 in all cases)

Table 3: Mean number of days to molt and the mean number of caterpillars that molted (±1 S. E.) on one week old foliage tested in 2011. (N=11 for 705316; N=12 for the remaining plants; Where D=*Populus deltoides;* N=*Populus nigra;* B=*Populus balsamifera;* T=*Populus trichocarpa;* M=*Populus maximowiczii*)

Plant	Cross	Days to Molt (Mean ± 1 SE)	Survival (Mean ± 1 SE)
3308	D x N	5.25 ± .12	9.25 ± .22
3565	D x N	5.42 ± .19	9.00 ± .25
P. tremuloides	*P. tremuloides*	5.50 ± .29	9.58 ± .19
3333	D x N	5.75 ± .49	9.00 ± .49
3230	T x D	6.08 ± .42	8.92 ± .73
915508	DN x M	6.25 ± .18	9.25 ± .18
3225	T x D	6.58 ± .36	9.08 ± .40
3729	N x M	7.92 ± .31	6.17 ± .88
915319	M x B	7.92 ± .75	8.83 ± .32
915313	M x B	8.75 ± .76	8.58 ± .65
3374	B x M	9.58 ± .60	8.42 ± .47
915320	M x B	9.92 ± .63	8.25 ± .46
750316	M x T	10.36 ± .90	6.00 ± .99
505508	M x DT	10.50 ± .89	6.25 ± .72
3389	D x B	10.75 ± .66	3.83 ± .69

Table 4: Preference Rank, Survivorship and Days to molt for clones in 2010 and 2011. (Consumption in 2010 is classified as follows (in cm^2): Low Consumption=[0, 0.1]; Medium Consumption=]0.1,0.5]; High Consumption=]0.5,2]; Consumption in 2011 is classified as follows (in cm^2): Low Consumption=[0,0.4]; Medium Consumption=]0.4,0.5]; High Consumption=]0.5,2]; Survivorship is classified as follow: Low Survivorship=[0,5], Medium Survivorship=]5, 8]; High Survivorship=]8 and above]; Days to Molt (DM) is classified as follows: Low DM=[0,7]; Medium DM=]7, 10]; High DM=]10 and above]; All values and number of replicates are based on Figures 1, 2, and Table 3)

Plant	Cross	2010 Preference Rank	2011 Preference Rank	Survival	Days to Molt
Populus tremuloides	*Populus tremuloides*	High	High	High	Low
131	DxN	-	High	-	-
3308	DxN	-	High	High	Low
3333	DxN	Medium	High	High	Low
3565	DxN	High	High	High	Low
3225	TxD	Medium	High	High	Low
3230	TxD	Medium	High	High	Low
915313	MxB	-	Medium	High	Medium
915319	MxB	Medium	High	High	Medium
915320	MxB	-	Low	High	Medium
3374	BxM	-	Medium	High	Medium
915508	DNxM	High	Medium	High	Low
916401	DNxM	Medium	-	-	-
505508	MxDT	-	Low	Medium	High
750316	MxT	-	Low	Medium	High
3729	NxM	Low	Low	Medium	Medium
3389	DxB	-	Low	Low	High

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APPENDIX A

EXPERIMENTAL PROTOCOL

Hybrids received in 2010 (as described previously) were used to record leaf morphology. Once a week, all hybrid poplar clones being tested were measured for leaf length, width, petiole length and leaf wet weight (one leaf per clone, a different leaf each week). At the end of the experimental season (in June 2010), collected leaf samples were oven-dried for a period of 72 hours and again weighed in order to calculate moisture content (where moisture = wet weight – dry weight) (Figure 3.1). Further, data were also collected for *P. tremuloides* (where *P. tremuloides* is the control plant; from April 19th to the 1st of June, one leaf per week from a random tree in either Saint-Esprit (45°91N, 73°65W) or Angrignon park). Experiments took place between April and June 2010: foliar data was gathered for clones 131, 3225, 3230, 3308, 3333, 3374, 3565, 3570, 3585, 3729, 915005, 915302, 915313, 915319, 915508 and 916401 as well as *P. tremuloides*.

In 2011, 16 of the clones received from Ripon were used to evaluate foliar morphology. In 2010, since a different leaf was chosen for measurement every week, leaf size data was subject to large variation and contained non-increasing values. To avoid this problem, two buds on one plant per clone were selected and followed in 2011: leaf measurements were taken on the same tagged buds. Measurements were taken every 10 days for most clones. After leaf-out, foliar morphology (leaf length, width, and petiole length) was evaluated on the leaves emerging from the tagged buds. *Populus tremuloides* foliage was collected at the Parc Nature du Bois-de-Liesse

(Pierrefonds, Québec). Whole branches were collected and stored in a water filled jar until needed.

In 2011, in most cases, the buds tagged for study did not leaf out. Untagged buds – those that were not measured - did leaf out, but no new buds were tagged and data was gathered only for those buds that were both tagged and that leafed out. The data on unbroken buds were discarded. Statistical tests were done on data available for those clones that leafed out.

Leaf out is defined as the presence of at least one fully expanded leaf. Different plants of the same clone leafed out on different dates, and some plants failed to leaf out at all (presumably dead). Recorded leaf out dates for each clone represent the sapling that leafed out first.

STANDARDIZING LEAF AGE

Because individual saplings leafed out on different days, measurements were taken on leaves of different ages. Leaves were therefore classified in bins representing a ***range of leaf ages***. Different bin sizes were used in different statistical tests; the range was 6 to 8 days.

STATISTICAL ANALYSIS

Correlations were used to establish relationships between leaf traits. Spearman correlations were used when the data set did not comply with the assumptions of the Pearson test. Gathered data

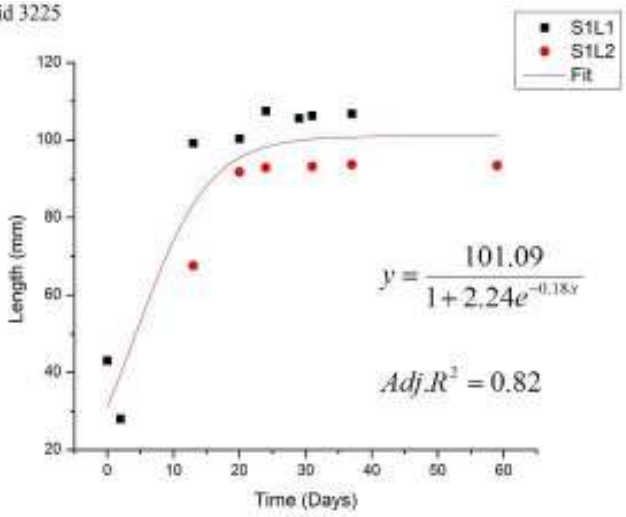
was analyzed using SPSS (version 16.0). Growth models were generated for leaf length and width using OriginPro v8.5.

RESULTS

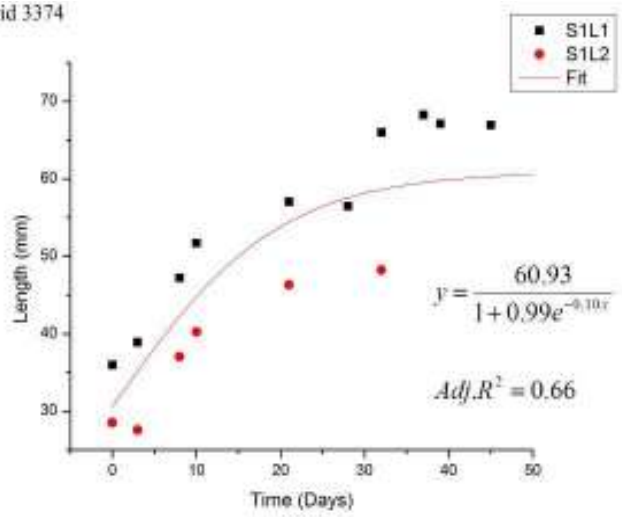
Correlations show that leaf measurements co-vary (Figure A3, Figure A4). Leaf length, width, and petiole length were found to be correlated to each other in both years (2010 and 2011). Moreover, petiole length was found to be correlated to the rectangular leaf area (in 2011). Wet mass was found to be positively correlated to leaf length, leaf width and petiole length. Further, moisture was found to be correlated to wet mass, leaf width, leaf length as well as petiole length.

Growth models show that most clones reach a mature size around 15 days with a few exceptions (Figure A1, Figure A2). Clones 3374 (BxM), 915311 (MxB), 915 318 (MxB), 915508 (DNxM), and 915320 (MxB) show a longer growth period - around 30 days - to reach maturity. Crosses between *P. maximowiczii* and *P. balsamifera* were shown, in the present study, to permit survival of first instar *M. disstria* larvae, but exhibited intermediate to high levels of resistance to late instar *M. disstria* larvae (Ralph, 2012). This difference might be linked to the slow growth of leaves and appearance of defensive traits in mature leaves only.

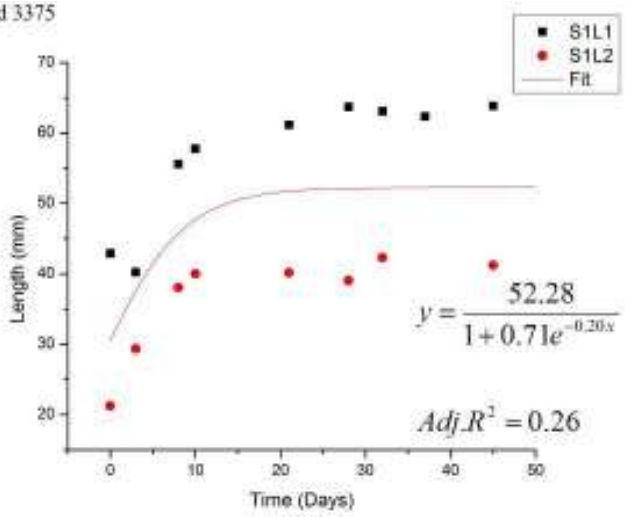
Hybrid 3225



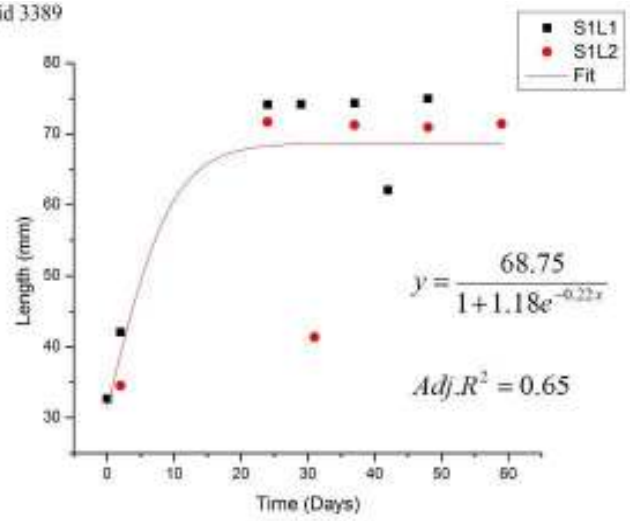
Hybrid 3374



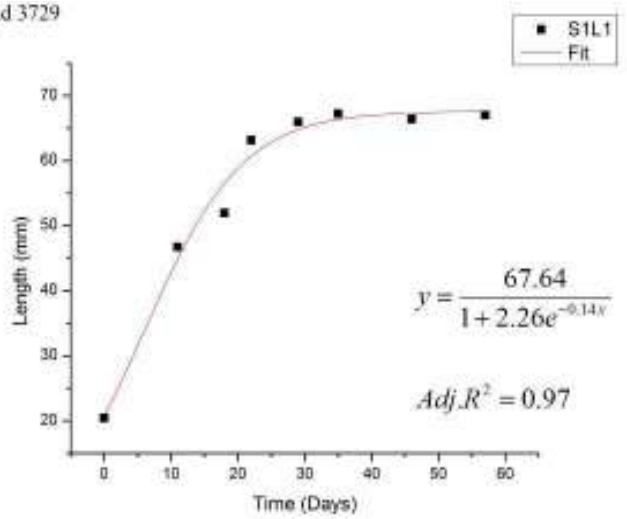
Hybrid 3375



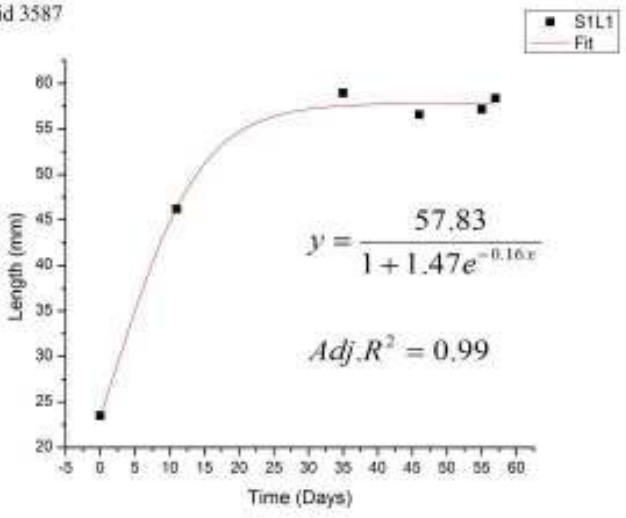
Hybrid 3389



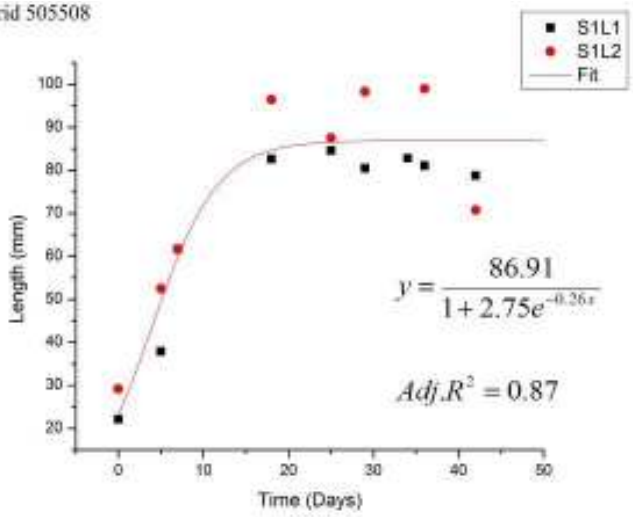
Hybrid 3729



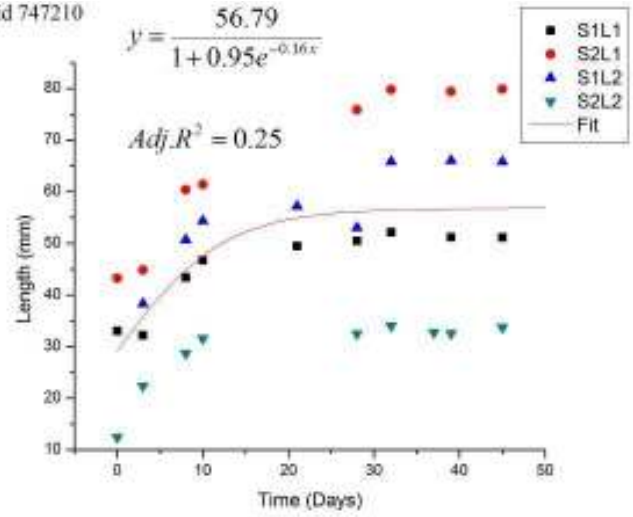
Hybrid 3587



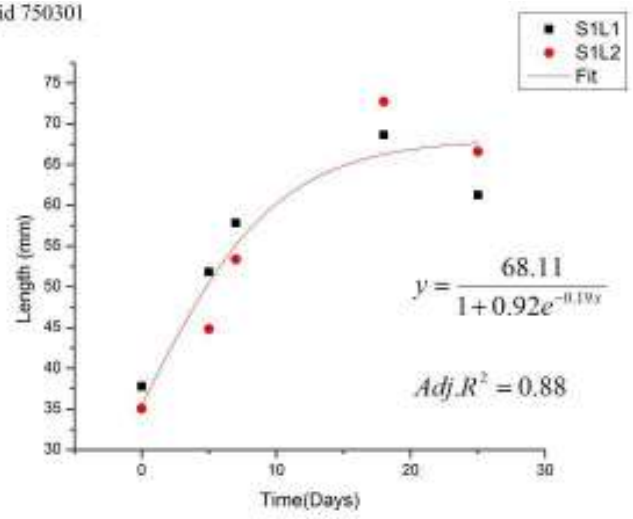
Hybrid 505508



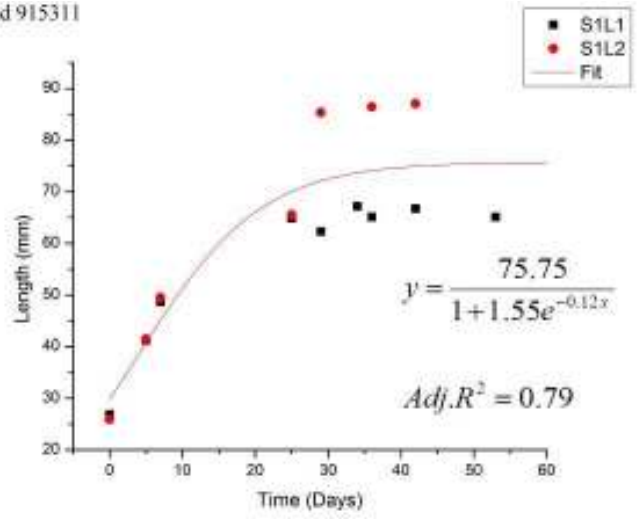
Hybrid 747210



Hybrid 750301



Hybrid 915311



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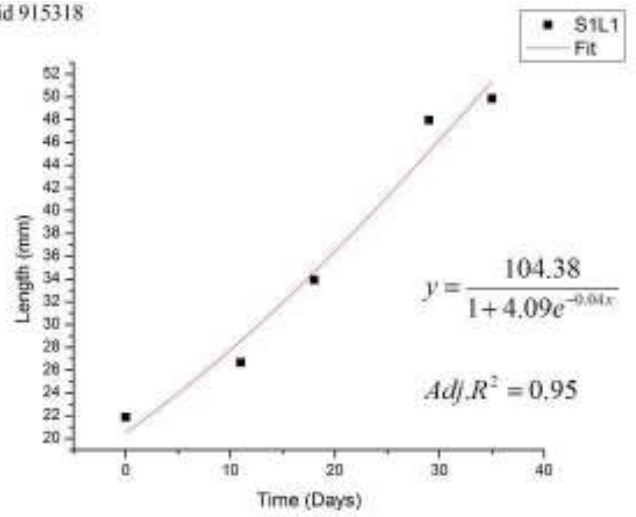
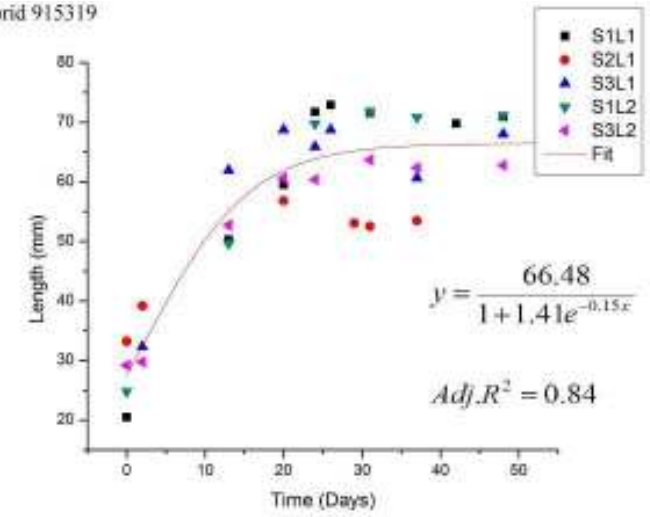


Hybrid 915319



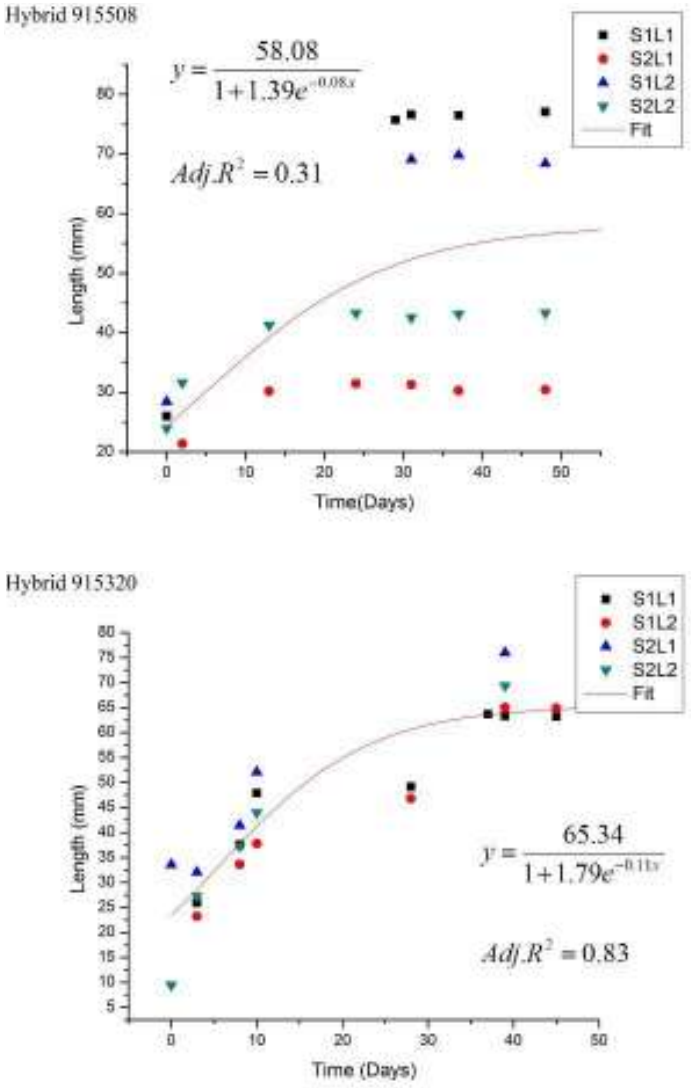
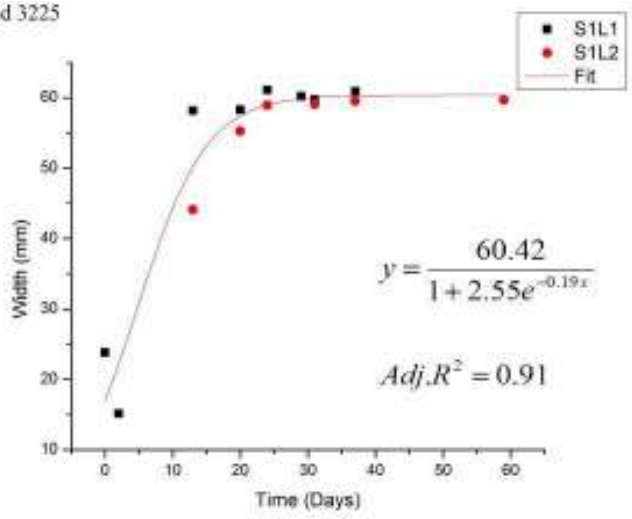
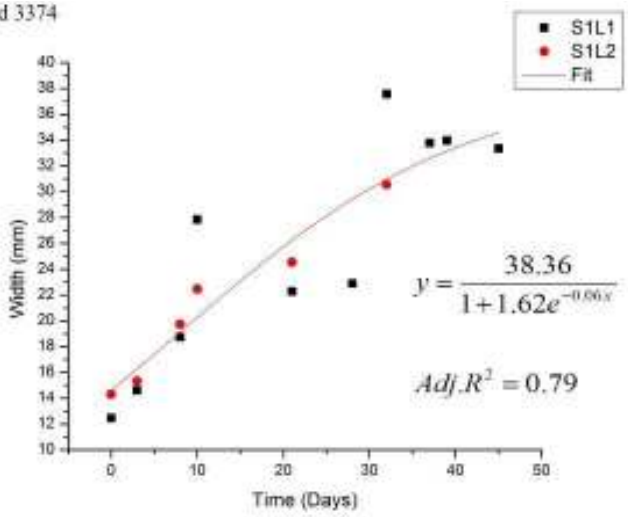


Figure A1: Length - Growth Rate of Leaves in 2011 (fitted to y = a/(1+k*exp(-b*t)). (Abscissa = Days after X where X is the 3rd May [clones 3374 (n=2), 3375 (n=2), 747210 (n=4)], 6th May [clones 915311 (n=2), 505508 (n=2), 915320 (n=4), 750301 (n=2)], the 11th May [hybrids 915319 (n=5), 3389 (n=2), 915508 (n=4), 3225 (n=2)] and the 13th May for clone 3729 (n=1), 3587 (n=1), 915318 (n=1); S=Sapling, S1=Sapling 1, S2=Sapling 2, S3= Sapling 3; L=Leaf, L1= Leaf 1, L2= Leaf 2)

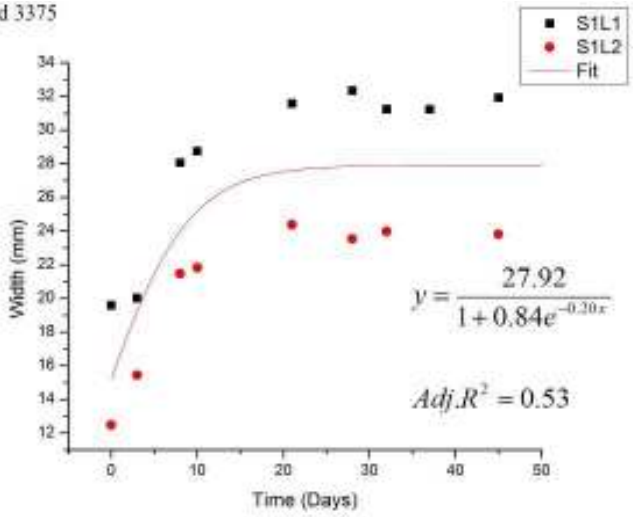
Hybrid 3225



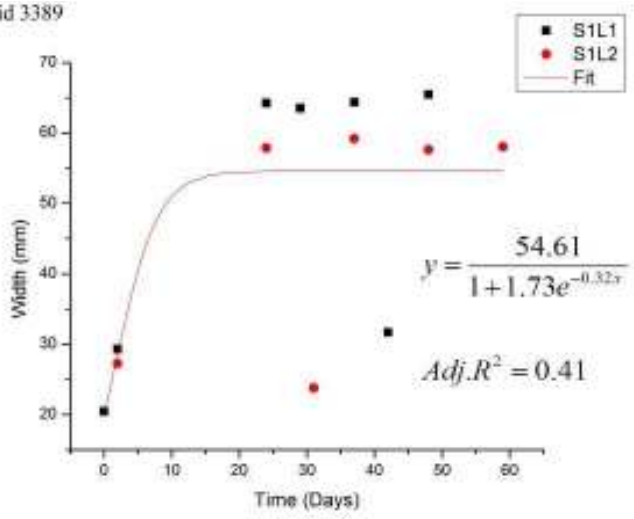
Hybrid 3374



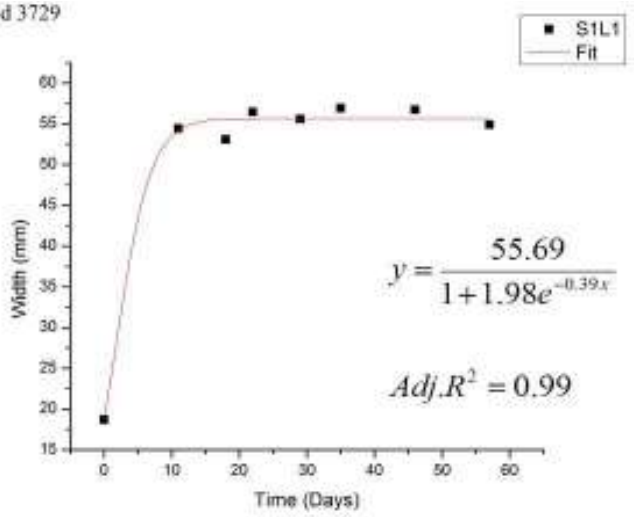
Hybrid 3375



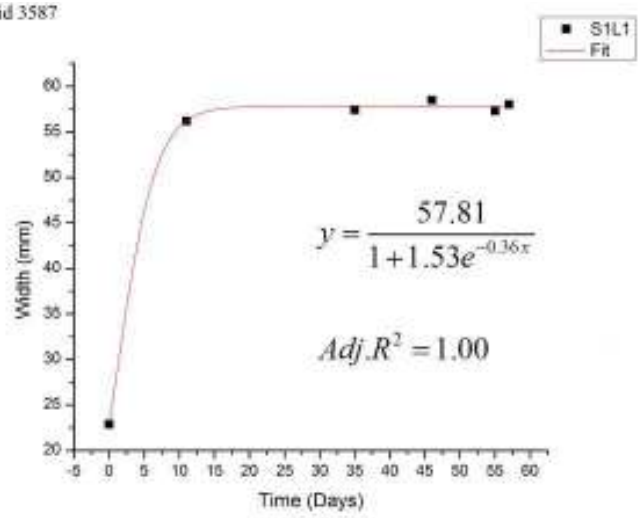
Hybrid 3389



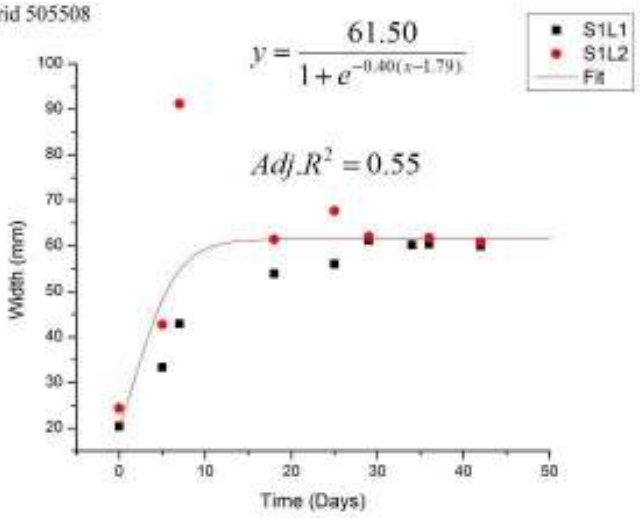
Hybrid 3729



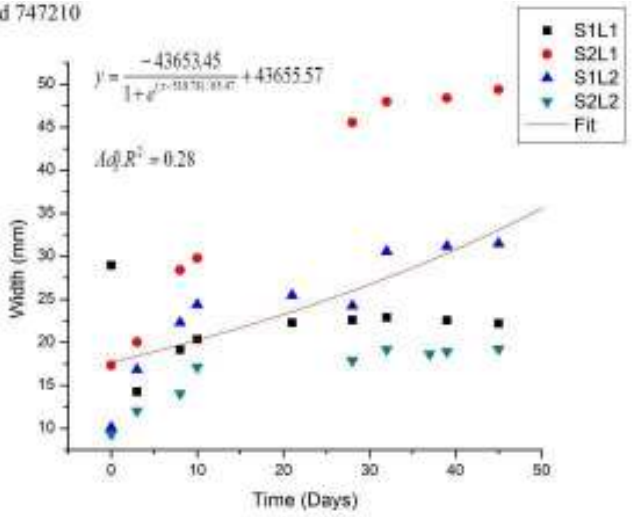
Hybrid 3587



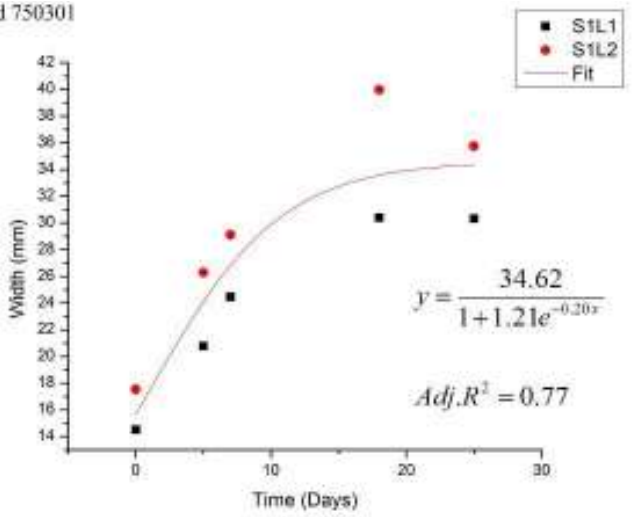
Hybrid 505508



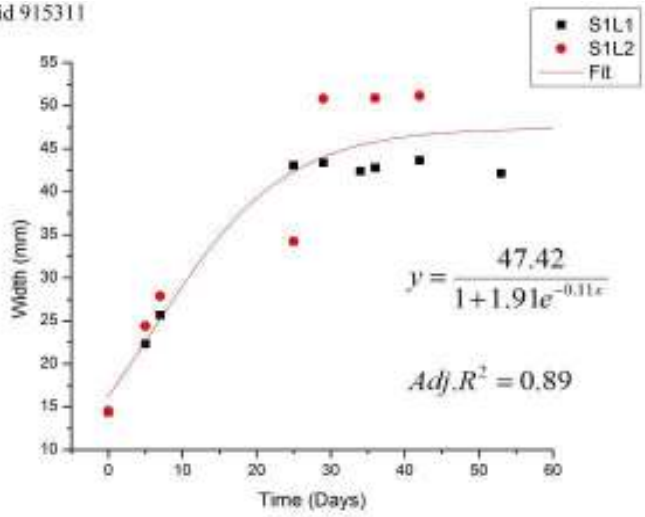
Hybrid 747210



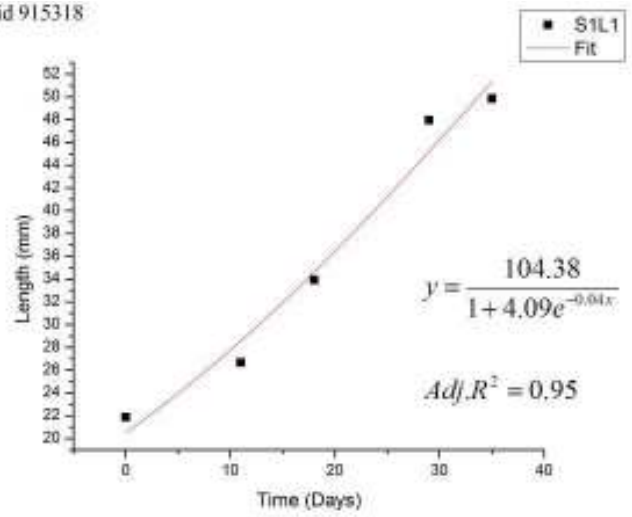
Hybrid 750301



Hybrid 915311



Hybrid 915318



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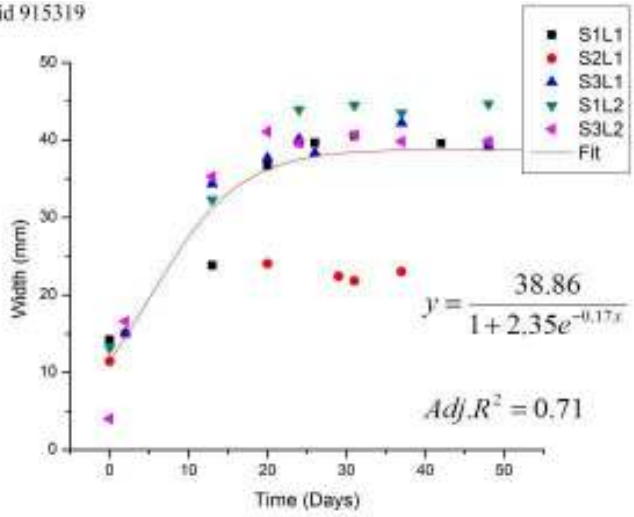

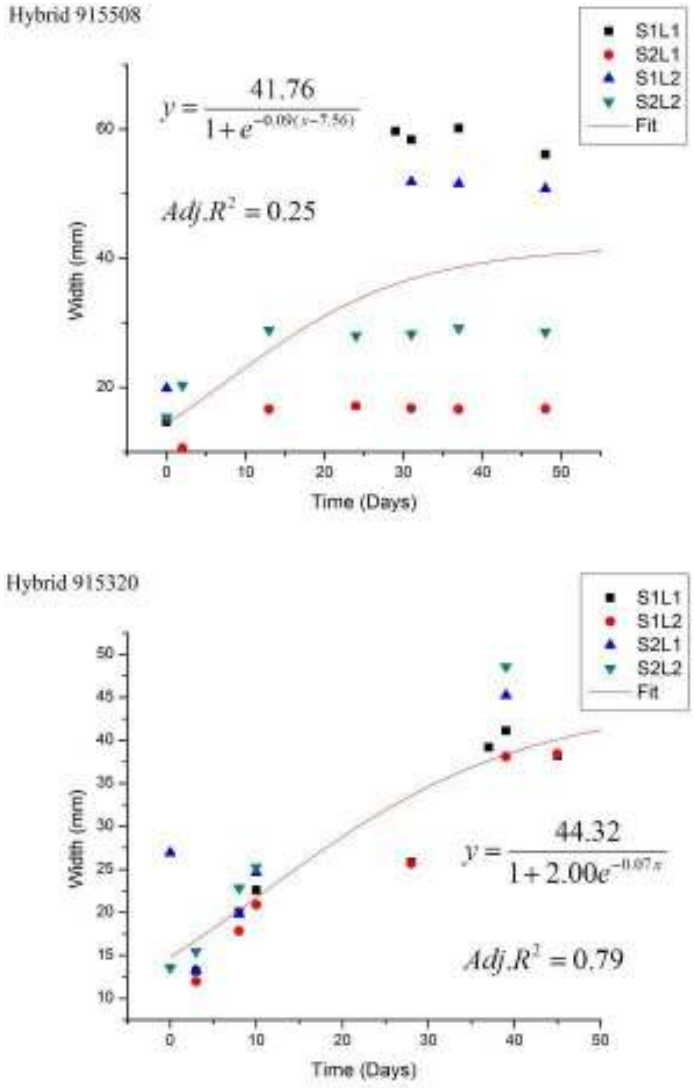

Figure A2: Width - Growth Rate of Leaves in 2011 (fitted to y = a/(1+k*exp(-b*t)), y = a/(1 + exp(-k*(x-xc))) except for clone 747210 which was fitted using the Boltzmann equation - y = A2 + (A1-A2)/(1 + exp((x-x0)/k))). (Abscissa = Days after X where X is the 3rd May [clones 3374 (n=2), 3375 (n=2), 747210 (n=4)], 6th May [clones 915311 (n=2), 505508 (n=2), 915320 (n=4), 750301 (n=2)], the 11th May [clones 915319 (n=5), 3389 (n=2), 915508 (n=4), 3225 (n=2)] and the 13th May for clone 3729 (n=1), 3587 (n=1), 915318 (n=1); S=Sapling, S1=Sapling 1, S2=Sapling 2, S3= Sapling 3; L=Leaf, L1= Leaf 1, L2= Leaf 2)

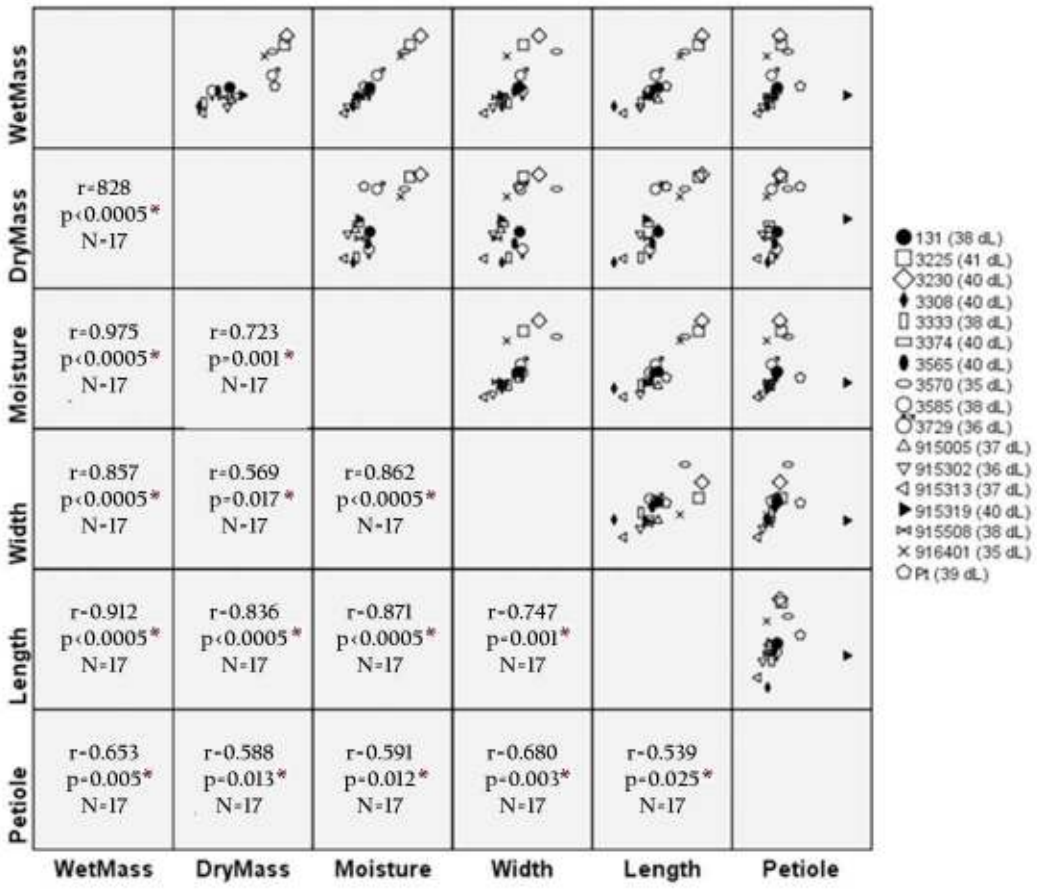

Figure A3: Spearman Correlation between Leaf Wet mass (g), Leaf Dry Mass (g), Moisture (g), Leaf Length (mm), Leaf Width (mm) and Petiole Length (mm) observed in 2010. (N_{total}=17; N=1 per clone for 17 clones; where dL=days after leaf out; dL system was used; *=significant at α=0.05)

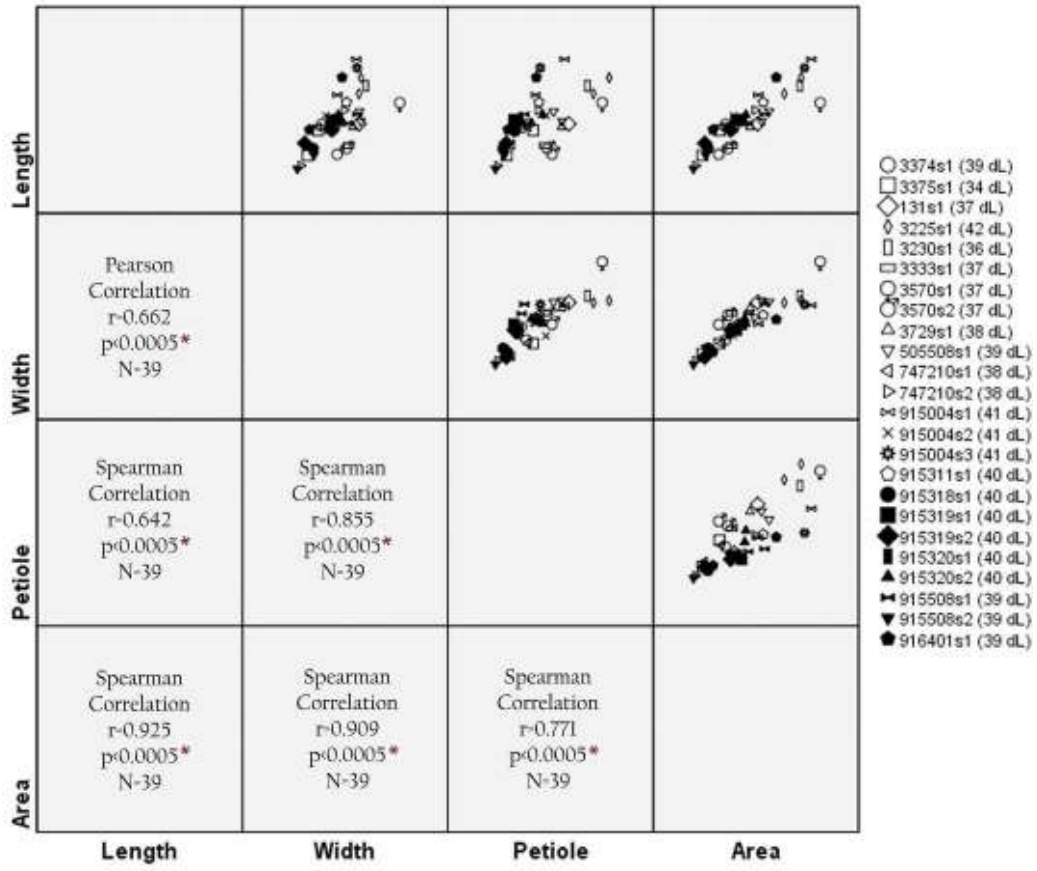

Figure A4: Correlation between leaf length (mm), width (mm), petiole length (mm) and rectangular area (length*width, mm^2) of late foliage in 2011. (S= sapling, s1=Sapling 1, s2=Sapling 2, s3=sapling 3; dL=days after leaf out; N=1 for clones 3374, 131, 3230, 3333, 3729, 915318; N=2 for clones 3225, 3375, 505508, 915311, 916401; N=3 for clone 3570; N=4 for clones 747210, 915004, 915319, 915320 and 915508; N_{total}=39; dL system was used; *=significant at α=0.05)