Effects of an early season heat wave on ecophysiological parameters related to productivity in Sugar Maple (*Acer saccharum* Marsh.)

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Submitted in partial satisfaction of requirements for the degree of Master of Science in Forestry

Faculty of Forestry
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Abstract:
Anticipated increases in the frequency, duration or intensity of high temperature events ('heat waves') have the potential to significantly impact forest form and functioning, but these events remain virtually unstudied in forest ecosystems. This thesis presents the results of an event-driven research effort into the impacts of three days of record-setting high temperatures in late May 2010 on key ecophysiological parameters in Sugar Maple (*Acer saccharum*. Marsh). High temperatures reduced photosynthetic capacity by ~66% versus previous years and total end-of-season leaf litter production by ~33% versus 2009. It is predicted that these reductions substantially reduced productivity for Sugar Maple in 2010. These results constitute the first description of the impacts of a short-duration heat wave on productivity-related parameters in a temperate forest tree. The predicted increase in high temperature events could make such impacts a significant, though so far overlooked, pathway of climate change impacts on temperate forests.
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This thesis is dedicated to Dr. Adele Crowder - inspiration

and in memory of Dr. Christopher Crowder - financier
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Full Length Abstract

Anticipated increases in the frequency, duration or intensity of high temperature events ('heat waves') have the potential to significantly impact forest form and functioning. Although the impacts of long (months or more) high temperature events co-occurring with severe precipitation deficits (drought) have received some attention, possible effects of short duration heat waves on forests remain virtually unstudied. This thesis presents the results of an event-driven research effort into the impacts of three days of record setting high temperatures in late May 2010 on key ecophysiological parameters in Sugar Maple (Acer saccharum, Marsh). High temperatures occurred before leaves were fully expanded, causing widespread leaf scorching and leaf abscission in Sugar Maple and other associated species throughout late May and June. Leaf abscission on high temperature days may represent a physiological attempt to control evapotranspirative stress induced by high temperatures. Leaf loss was higher in mature canopy trees (33.8%) than in saplings (19.2%). Photosynthetic capacity (A_max) in mature trees was reduced by ~66% compared to previous years (2002-2006) but had recovered to near-normal levels when re-sampled in late July/early August. Trees reflushed (produced neoformed leaves) to replace lost preformed leaves. Neoformed leaves displayed morphological characteristics associated with acclimation to high temperatures, but enhanced photoprotective properties (altered pigment concentrations) were not observed. Capacity to reflush was much larger in mature trees than in saplings, probably due to increased reserves of non-structural carbohydrates. Despite reflushing total end of season litter production for Sugar Maple (all age classes) was reduced by ~33% versus the only prior year for which data are available (2009). Depressed photosynthetic capacity throughout
the summer months combined with substantial reductions in leaf production is predicted to have substantially reduced productivity for Sugar Maple in 2010. The extent to which other species compensated for this reduction at the landscape scale is unclear. Ecophysiological vulnerability (low heat stress tolerance) is suggested to explain the severe response observed in Sugar Maple, but the role of leaf ontogenetic stage in determining the magnitude of leaf loss requires further study. These results constitute the first description of the impacts of a short-duration heat wave on productivity-related parameters in a temperate forest tree. The predicted increase in high temperature events could make such impacts a significant, though so far overlooked, pathway of climate change impacts on temperate forests.
Chapter 1: Introduction and Literature Review

1.0 High Temperature Events in a Changing Global Climate

Current evidence for anthropogenic global warming is unequivocal (IPCC 2007), and expected impacts and adaptive strategy are a major area of concern for forest managers and ecologists. Implicit in predictions of global warming is a "likely" (>66% chance) increase in the frequency, duration or intensity of extreme high temperature events or heat waves (Karl & Trenberth 2003; Meehl & Tebaldi 2004; IPCC 2007). This can be conceptualized as the result of an upward shift (with some distortion) of the two-tailed frequency distribution of temperature (WGI 2007). While the distribution of heat waves is spatially heterogeneous, current models predict a disproportionate increase in heat wave frequency in the Northern Hemisphere (Meehl & Tebaldi 2004; WGI 2007), suggesting that understanding the impacts of high temperatures on northern temperate forests should be of special importance.

To date, observational studies have linked high temperature events to temperate forest functioning through induced or co-occurring medium or long term droughts (Ciais et al. 2005; Rennenberg et al. 2006; Carnicer et al. 2011), but no studies address the impacts of short (<2 wk) high temperature events in the absence of water stress. Because of this focus on longer term high temperature stress no work has yet investigated the role of forest phenology in determining high temperature impacts, although strong relationships between leaf age and leaf stress susceptibility have been proposed (Coleman 1986). Thus a recent global review of high temperature-induced forest mortality (Allen et al. 2010) found 22 cases of large-scale tree mortality from Europe and North America in
which heat waves or warming and drought were suggested as the key driving factors, but only 2 studies in which warming alone was proposed to cause mortality. Of these one (Beier et al. 2008) found root kill due to winter freeze/thaw to be responsible for observed mortality, while the other (van Mantgem et al. 2009) concluded that high temperatures had adversely affected the landscape hydrologic balance. Similarly, although some studies have addressed seasonality in heat or warming (e.g. Nelson & Thomas, *in review*), none address the importance of the specific phenological stage in which heat occurs. It is clear that the impact of short-term, extreme high temperature events (hereafter "heat waves") on forests and their potential interactions with forest phenology remain largely unexplored.

1.0.1 Event-Driven Research and the Role of Inference

Ecological systems are often large, complex, and dynamic (Hilborn & Ludwig 1993). Causality in these systems can be hierarchical or overlapping, and an observed ecological phenomenon can be the product of multiple interacting causes (Hilborn & Stearns 1982; Holling & Allen 2002). Consequently reductionist experimental studies, traditionally focused on the validation of a single explanation, may not be adequate for analyzing complex ecological causality (Plowright et al. 2008). Even where appropriate, such studies are limited by practical limits to replication (cost, ethical considerations, replication time), experimental control and randomization that may prove impossible to surmount (Hilborn & Ludwig 1993; Plowright et al. 2008). These limitations, particularly the inability to impose controls, may also preclude the use of more flexible systems of inquiry such as strong inference (Platt 1964). Droughts, heat waves, and frost damage are
all examples of forest disturbances for which large scales, complex interacting factors, and difficulties in replication might be expected to preclude the elucidation of causality through traditional methods of inquiry.

Since large-scale ecological phenomena may not be replicable, and smaller scale experimental simulations may fail to include all potential causal factors, it is critical to take advantage of opportunities to study such phenomena when they occur naturally—a research approach best characterized as 'event-driven'. Because event-driven phenomena are not tractable to experimental manipulation, ecologists must use observational studies to infer causality (Plowright et al. 2008). This is true even when individual potential causal factors are distributed across a wide range of naturally occurring 'treatments', because investigators have no way of knowing that treatments were applied at random with respect to naturally occurring site differences (Hilborn & Ludwig 1993). However, relying on observational inference does not mean abandoning rigorous standards of truth, and attempts have been made to define comprehensive criteria by which causality may be inferred in observational studies. In this regard Plowright et al. (Plowright et al. 2008) offer a modified version of Hill's Criteria (Hill 1965), originally formulated for use in the field of epidemiology and applied by these authors to studying the origins of disease in natural systems (Table 1). Note that not all criteria must be met to establish causality, and that other such rubrics exist. This modified version of Hill's Criteria is presented here because it represents a comprehensive inductive framework that illustrates the criteria by which causality in complex ecological phenomena may be inferred.

The criteria presented in table 1 are not a generally accepted standard by which causality may be inferred in complex ecological phenomena, but in the absence of such a
guide they constitute a powerful framework for researchers engaged with event-based phenomena. However, when applying these criteria, it is necessary to consider the nature of the causal factor or factors. Hilborn and Stearns (1982) argue that there are three fundamental patterns of causality. A phenomenon may have i) one cause which is "necessary and sufficient" (Hilborn & Stearns 1982:162), ii) several sufficient causes, none of which is necessary or iii) several necessary causes, none of which is sufficient. Work examining Sugar Maple decline in the context of the Multiple Stress Hypothesis (section 1.7.2) provides a good example of an observed phenomenon displaying type iii) causality. The literature on high temperature stress and induced drought (section 1.4) suggests that high temperature stress is capable of exerting significant impacts on forests even when acting in isolation (type i causality), although it is clear that secondary factors affect the magnitude, severity and characteristic of effects. However, any analysis of the effects of high temperature stress must carefully consider whether the observed impacts can be entirely explained without reference to other casual factors, as falsely attributing type i) causality will overlook important contributing factors.

Studies on drought impacts are one of several fields in which criteria analogous to one or more of those presented in table 1 have been used to infer causality. Due to the complex interaction of multiple ecological factors causality in landscape-level drought impacts must be inferred rather than experimentally tested, and thus "quantitative assessments of the triggers and potential extent of drought-induced vegetation die-off remain pivotal uncertainties in assessing climate-change impacts" (Breshears et al. 2005:15144). However, management demands assessment, and despite quantitative uncertainty strong qualitative linkages can be made between observed environmental
phenomena and observed impacts. Thus droughts have been linked to vegetation (including forest) die-off by the criteria of plausibility, specificity, biologic gradient, consistency, coherence and temporality (e.g., Allen & Breshears 1998; Breshears et al. 2005). Other studies on environmental phenomena are often less rigorous- for example, altered pollen production has been attributed to the 2003 European Heat Wave solely on plausibility, consistency, coherence and temporality (Gehrig 2006), and changes in spring flowering dates in Alberta have been linked to the Southern Oscillation Index based on plausibility, specificity, consistency, coherence and temporality (Beaubien & Freeland 2000). Further use of qualitative associations to infer cause can be seen in such disparate research areas as attempts to determine the impacts of late frost on tree ring radial growth (Dittmar et al. 2005) and almost the entire literature on Sugar Maple decline (section 1.7).

It is important to note that in these studies ambiguity in the relative magnitude of secondary versus primary factors does not preclude analysis of primary factor impacts. As all these examples investigate environmental phenomena, unique weather histories and specific phenomenological conditions will inevitably confuse attempts to unambiguously isolate single causal factors. Regardless, critical use of comprehensive inferential criteria allows scientists to draw conclusions about the impact of observed complex large-scale ecological phenomena in the absence of traditional reductionist hypothesis testing to determine causality.

1.0.2 Scope of this Chapter

Despite the lack of large-scale in situ studies of the effects of acute heat events on temperate forests, an investigation into this topic can draw on a large body of related
literature. Plant responses to high temperature stress have been well studied from molecular to organismal scales, and numerous experimental studies assess the impacts of moderate warming at more complex organizational scales (Norby et al. 2007). Co-occurring heat and drought events have also been the focus of a substantial research effort (Allen et al. 2010) and due to biophysical linkages between these two abiotic stresses (section 1.2.1) some of these results are applicable where high temperatures act in isolation (Wahid et al. 2007). Similarly, work on ontogenetic differences in tree response to stress and on within-canopy gradients in photosynthetic function can be used to predict how acute heat impacts would manifest within a mixed-age, mature forest. This chapter reviews these theoretical areas, drawing on experimental studies and review papers to summarize what is known about temperature forest response to high temperature events. Where possible the discussion is focused on the study organism of this thesis (*Acer saccharum* Marsh.). Note that for most of the topics reviewed here more comprehensive summaries exist (e.g. for molecular and cellular effects of high temperatures in plants, see Berry & Bjorkman 1980; Georgieva 1999; Wahid et al. 2007), and in many cases research into the subject matter is both ongoing and contentious. It should therefore be understood that this work does not constitute an authoritative review, and serves only as a necessary contextualization to the associated original research.

1.1 Heat and Drought

While field trials and laboratory experiments aiming to elucidate the effects of short-term temperature shocks are extremely scarce, warming studies and the substantial body of knowledge dealing with drought effects often provide key information about the
processes by which trees can be affected by high temperature events. Although the magnitude (and sometimes the direction) of tree response observed in these studies cannot always be generalized to include the impacts of high temperatures, key physiological impact pathways, changes in stand structure, and effects on macro-parameters of ecosystem functioning (e.g. biodiversity) can be identified. Combining these generalizations with what work does explicitly consider the effects of high temperature events acting in isolation, it is possible to construct a reasonably coherent picture of the probable effects and mechanisms of high temperature stress on the conditions and functioning of trees and forests. Studies examining the effects of drought (as well as heat) are incorporated into sections 1.2, 1.3 and 1.4.

The highly interrelated nature of heat and drought events poses a challenge to any investigator primarily interested in the former. Given that heat can induce a condition of water stress through evaporative and evapotranspirative forcing on the water balance of landscapes and plants (Allen 2009), it would seem necessary to establish some threshold of water availability past which heat impacts can be considered to act more or less in isolation. Of course, such thresholds would be expected to be both site- and species-dependent, and their elucidation could fairly demand an independent research effort. Such thresholds would also be fairly artificial, as in the natural world high temperature stress and water stress tend to co-occur to the extent that they are often discussed together as a single stress factor (e.g. Roualt et al. 2006; Boisvenue & Running 2006). Since establishing a workable threshold of this type is beyond the scope of this study, this chapter will consider the literature relating to forests and drought to be relevant to the subject of high temperature stress inasmuch as i) it deals with drought effects than can
clearly be induced by short-term high temperatures such as those observed in the May 2010 heat wave, or ii) is sufficiently general to cross-apply, and represents an area in which no studies on the effects of high temperatures alone are available.

1.2 Tree-level Impacts of High Temperatures

At the level of the individual tree, the effects of heat can be seen as a combination of molecular processes, contingent physiological responses, and their interaction with the immediate (proximate) environment. Although most evaluations of heat impacts on trees do not consider temperature fluctuations in isolation from vapor pressure deficit and soil water availability, temperature alone can have significant effects on tree condition and functioning, ranging from effects on productivity (section 1.4.2) to damage, recovery and biochemical alteration to tissues (Bassow et al. 1994) to tissue necrosis or whole-tree mortality (Allen et al. 2010). When high temperatures co-occur with water deficits or high light these effects tend to be exacerbated (e.g. García-Plazaola et al. 2008). The focus in this section will be on physiological processes; here the interactions with high temperatures are usually mediated by water supply. The best understood mechanism of heat-related damage in trees is xylem cavitation, described in section 1.2.1; other impacts are reviewed in 1.2.2.

1.2.1 Air-Soil Linkages and Xylem Cavitation

Most available work on the subject of high temperature impacts on trees draws on drought stress literature, and is primarily concerned with the effects of increased evapotranspirative rates. The saturation vapor pressure of air increases with temperature,
resulting in a higher vapor pressure deficit (VPD) and consequently greater evapotranspirative stress; by this mechanism high temperatures can create an 'induced drought' at the level of the individual plant, stand or landscape (Allen et al. 2010). Soil water and air temperature are thus coupled by water columns in vessel elements extending from the soil-root interface, with rates of exchange mediated by the spatial extent and density of root system, its uptake efficiency and volumetric soil water content, and by the leaf-air interface, where surface roughness, stomatal control, VPD and windspeed regulate exchange (Breda et al. 2006). Along with non-plant-mediated evaporative stress, this fundamental linkage is responsible for the intertwining of heat and drought impacts at whole-plant and greater scales. It also provides the primary mechanism for heat damage to individual trees: severe evapotranspirative stress may increase negative pressure within vessel elements until hydraulic connectivity is lost due to embolism (Tyree & Sperry 1989), a phenomenon known as xylem cavitation. The primary mechanism of defense against xylem cavitation is stomatal closure, but when evapotranspirative stress is prolonged this strategy may lead to carbon starvation, severely weakening trees (Allen et al. 2010). In addition to stomatal closure, or other methods of embolism avoidance, trees may deal with the problem of xylem embolisms by refilling embolized vessel elements or by producing new xylem, also a carbon-intensive solution (Nardini et al. 2011). Mechanisms of embolism reversal (refilling of gas-filled vessel elements) are not yet fully understood, but may be driven by active loading of embolized cells with water and solutes passed through ray cells from the phloem (Nardini et al. 2011), a process made possible by the biochemical (and physical) isolation of embolized vessel elements from the transpiration stream (Holbrook and Zwieniecki...
1999). In some species (but not in the only *Acer* spp. tested, *A. negundo*) refilled xylem may be more prone to additional cavitation, a phenomenon known as cavitation fatigue (Hacke et al. 2001). How these repair processes and their impacts might be affected by high temperature stress is currently unknown. Recovery of xylem from seasonal (winter) cavitation has been shown in Sugar Maple, but observed recovery was most likely driven by positive root pressures following the spring thaw (Sperry et al. 1988).

### 1.2.2 Other Known Effects

In section 1.2.1 high temperature effects were presented as the outcomes of molecular processes, physiological responses, and environmental conditions. Though true, this is something of an oversimplification. The effects of high temperatures on the individual tree *in situ* are the product of the interaction of temperature stress with the past and present environment, tree health, and the future occurrence of other stress factors (see section 1.7.2 for an explanation of the multiple stress hypothesis, a formal treatment of this idea concerned with the specific outcome of tree mortality). For example, Auclair et al. (1996) argue that high temperature impacts are more severe when preceded by spring freezing events that reduce water transport ability (e.g. freeze killing of fine roots), since this increases evapotranspirative stress on remaining healthy tissue and promotes vessel cavitation. Lower soil temperatures also increase the viscosity of soil water, limiting hydraulic flow through roots (Brodribb & Hill 2000). Determining which post-temperature event impacts are attributable to heat alone is thus extremely difficult, but despite this caveat some outcomes can be identified in which high temperatures are the primary causal factor. Partial crown necrosis and whole tree mortality in Douglas Fir
(Pseudotsuga menziesii) has been attributed to heat wave-induced drought in France (Martinez-Meier et al. 2008). Archaux and Wolter (2006) review drought effects on forests, and conclude that short-term effects are reduced growth and fecundity and increased mortality, leading to lasting growth reductions, delayed mortality and increased susceptibility to pathogens and disease over longer time scales. According to these authors these outcomes have yet to be confirmed in field trials, but are strongly supported by our understanding of plant growth and reproduction. High temperature stress applied to trees in a condition of water abundance could cause similar effects through heat-induced physical damage and consequent productivity declines. Changes in growing season length, productivity and tree water balance have been observed following high temperature events (Boisvenue & Running 2006), although these effects have generally been investigated in regard to moderate and sustained warming. High temperatures can also impact leaves—indeed, as the active site of gas exchange between the atmosphere and trees/soil, leaves may be the first sites where the effects of temperature stress are felt.

1.2.3 Predisposing Factors

Given that xylem embolism is perhaps the major tree-level impact of high temperatures (and drought), it is unsurprising that factors predisposing trees to high temperature damage should often be related to the movement and exchange of water: leaves are of particular interest in this regard. It has been argued that greater LAI means greater drought sensitivity (Archaux & Wolters 2006), and that plants with large horizontal broadleaves are more susceptible to daytime overheating (Jordan & Smith 1995). Leaf shape may also play a role: Nicotra et al. (2008) examined leaf shape in eight
species of South African *Pelargonium* (the scented geraniums) and found that more lobed/dissected leaves had higher thermal optima and rates of carbon gain but also higher rates of water loss than more compact leaves. The proposed explanation is that more dissected leaves will have thinner boundary layers (zones of little or no air movement proximate to the leaf surface) and thus greater convective cooling compared to more compact leaf types. Whether leaf shape exhibits plasticity in response to high temperature stress in temperate trees is as yet unexplored, although reductions in LAI of 2-3 x have been observed in years following the year in which drought stress occurred in many species (Breda et al. 2006); of course, the magnitude of this response may be conflated with crown damage resulting from xylem embolism. Other factors predisposing individual trees to high temperature impacts are phenotypic: being located in shade or near the species range southern boundary (Archaux & Wolters 2006).

1.2.4 Acclimation and Vulnerability in Leaves

While relatively few studies have focused on physiological changes in mature leaves surviving high temperature stress, it is known that they have the potential to acclimatize. Substantial changes occur in the first 1-2 days following the onset of high temperatures, with most processes being complete in days or weeks (Berry & Bjorkman 1980). The initial dramatic response may be mostly attributable to reduced conductance from stomatal closure; another common response pathway to quickly ameliorate induced water stress from high temperatures is active leaf shedding (Breda et al. 2006). Even where leaf shedding does not occur, high levels of abscisic acid have been shown to occur following heat stress and are thought to be an important component in acquired
thermotolerance (Wahid et al. 2007). Damage to leaves surviving severe high temperature stress is often visible as scorching, leaf senescence, leaf abscission and growth inhibition; less visible effects include reduced cell size, mesophyll cell damage, and structural deformation of organelles (Wahid et al. 2007). Where trees bear preformed leaves effects may also exhibit a significant time lag, with the environmental conditions in the year of bud formation dictating the number of leaves expanding in the subsequent growing season (Steingraeber 1982). Changes in leaf thickness and leaf area duration have also been observed (Breda et al. 2006), and may be related to known alterations in carbon allocation (such as increased fine root growth) within plants following heat stress (Hanson & Weltzin 2000). In general, plants exhibiting these adaptive responses have lower productivity (Hanson & Weltzin 2000).

Work by Coleman (1986) suggests ontogenetic patterns in leaf vulnerability to biotic stress based on an allometric analysis (see section 1.5.1). The author argues that the ontogenetic transition between leaf status as a metabolite sink (developing leaves) and source (expanding or mature leaves; identifiable by a sudden and dramatic increase in sucrose levels) represents the most vulnerable stage in leaf development (for a review of the source-sink transition in leaves see Turgeon 1989). Young leaves are chemically well defended, while older leaves tend to be sclerophyllous, fibrous, and tannin-rich. At the source-sink transition leaves are both nutritionally adequate for predation and lacking in defensive attributes characteristic of older or younger leaves. A number of reviewed studies on pest and pathogen interactions with *Populus* spp. find experimental data supporting this sink-source hypothesis for maximum leaf vulnerability, but also highlight the ways in which heterogeneity in pest feeding strategies can overcome plant defenses
and concentrate damage at other ontogenetic stages. Interestingly, *Populus* leaves are found to also be most vulnerable to an abiotic stress (ozone) at the sink-source transition due to the maximization of the ratio of stomatal to leaf area. Although not shown to be applicable to other species, maximization (if present) would suggest increased vulnerability to heat stress at this ontogenetic stage.

1.3 Stand Level Impacts of High Temperatures

The impacts of high temperature events at the stand scale are the product of tree-level responses, and thus the distinction between tree-level and stand-level impacts may seem rather arbitrary. However, the responses of individual trees to high temperature events produce important shifts in cohort structure and community assemblages that are not apparent at lower organizational levels. These shifts can have profound impacts on both ecosystem functioning and forest management goals, as natural processes such as tree mortality (Allen et al. 2010) can be greatly accelerated due to temperature stress while even brief exposures to high temperatures during seed filling can diminish seed number and viability and delay germination (Wahid et al. 2007). Increased mortality rates and reduced recruitment can drive changes in cohort structure, while interspecific differences in tree response would be expected to result in altered community composition at the stand scale. Although the role of high temperature stress in altering community composition through seed damage has yet to be the subject of comprehensive experimental inquiry, differential seedling mortality has been well established between species. For example, interspecific differences in seedling mortality in *Quercus* spp. and *Acer* spp. (Huddle & Pallardy 1996) have been observed following extreme soil heating.
In general, both very old and very young individuals of most plant and animal taxa are most sensitive to drought events (Archaux & Wolters 2006). Available data do not permit a similar generalization for the effects of warming. Dendrochronological studies have found age differences in tree growth response to climatic variability (Szeicz & MacDonald 1994; Linderholm & Linderholm 2004; Yu et al. 2008) but results are not consistent. Investigations with different species have found, variously, no significant relationship between tree age and climatic response (Esper et al. 2008), increased climatic sensitivity in older trees (Carrer & Urbinati 2004; Yu et al. 2008), increased sensitivity to unfavorable conditions in middle-aged trees (Linderholm & Linderholm 2004) and a relationship in some species but not in others (Carrer & Urbinati 2004). If an acute high temperature event induces drought at the stand scale, it would be expected to produce more even-aged stands (by reducing abundance of older and younger individuals), but in the absence of co-occurring water stress it is not yet known if age responses will be apparent. In either case, interspecies differences in temperature tolerance (Berry & Bjorkman 1980) would be expected to favor a shift in stand composition towards more heat-tolerant species (for example, Quercus spp. in Eastern North America).

Heat may also impact stand structure without causing tree mortality. Crown dieback may favorably affect recruitment for shade-intolerant species through changes in the understory light environment (Archaux & Wolters 2006); the situation is analogous to the effects of thinning treatments. Forest managers may thus take advantage of high temperature events to facilitate alterations in stand composition; in general, however, management practices are aimed at mitigating the impacts of high temperatures. Managers may thin stands to reduce competition, select for heat-resistant genotypes or
plant heat-tolerant species (Allen 2009), or may attempt to minimize LAI at the stand level to reduce the damage caused by xylem cavitation (Archaux & Wolters 2006). Other non-traditional management practices might aim at encouraging viable pollen distribution, as heat tends to be more deleterious to pollen than to ova (Wahid et al. 2007). However, as age-mediation of high temperature impacts on temperate forests is not yet fully understood, research on this fundamental aspect of stand response is vital for effective forest management.

1.4 Impacts of High Temperatures on the Forest Ecosystem

Key ecosystem parameters visible at the level of the entire forest ecosystem have been shown to exhibit strong responses to both drought and sustained warming, but the impacts of acute high temperature events remain generally unstudied and must largely be inferred from this related literature. This observation is increasingly valid at larger spatial scales, as conducting heat-shock experiments becomes increasingly less feasible with increasing size. As large, infrequent disturbances, high temperature events such as heat waves may be expected to act as catalysts for sudden and unpredictable changes in ecological systems, potentially abruptly exceeding unknown thresholds (Turner & Dale 1998). There is a growing awareness of the increasing risk to forests from heat- and drought-related stress, even in non-water-stressed environments (Allen 2009) and with the proliferation of powerful large scale monitoring techniques such as eddy covariance we may expect to see increasing attention paid to this important issue.

In the current state of relative ignorance about the impacts of high temperature events on forests it is still possible to make some general statements. Due to the highly
interrelated nature of heat- and water- stress the observation that preference for cool or wet habitats and the absence of ground reserves predisposes forest ecosystems to drought impacts (Archaux & Wolters 2006) is probably applicable to high temperature impacts as well. In this regard well-established relationships between plant species, their preferred growth environment and tolerance of high temperatures (see Berry and Bjorkman, 1986) also probably apply at larger scales. It would be reasonable to expect ecosystem community assemblages that have evolved in the relative absence of high temperatures to exhibit less resilience and adaptive capacity than those from areas more frequently subjected to this form of disturbance. Furthermore, due to the relatively slow nature of many forest ecosystem processes, multi-year delays in response would also be expected; such time lags have been observed following drought events (Allen 2009) and high temperature / drought events (Ciais et al. 2005).

Impacts of high temperature events at the ecosystem level may occur indirectly, and these indirect impacts may be greater than direct effects. This has often proved true following drought events, where secondary factors such as insect pests, pathogens and forest fires have often proved more damaging than initial dieback and productivity loss (Roualt et al. 2006; Archaux & Wolters 2006). Secondary factors further complicate predictions of heat effects, as individual factor responses may be heterogeneous and display delayed responses (e.g. Roualt et al. 2006). Terminal stress outcomes such as forest mortality may thus be the product of a complex set of interacting secondary factors related to an underlying abiotic stress (Allen 2009). Of course, indirect effects may also have positive outcomes: for example, Archaux and Wolters (2006) note that increases in deadwood following drought events may raise biodiversity.
1.4.1 Nutrient Cycling

Available data suggest that nutrient cycling in forest ecosystems is likely to be sensitive to acute high temperature events. Temperature is known to be a key factor regulating decomposition, N mineralization, nitrification and denitrification, methane emission, fine root dynamics and plant nutrient uptake in undisturbed ecosystems (Norby et al. 2007); the importance of this regulatory role depends primarily on whether the effects of temperature are diminished by the impacts of some other stress factor. Changes in resource use and phenology due to temperature can be expected to alter availability of key nutrients, ultimately impacting productivity. Rennenberg et al. (2009) review the available literature and conclude that N-limited forests are generally characterized by a closed (internal) N-cycle at the ecosystem level, with spring N demand being met by N storage accumulated the previous fall. High temperature-induced changes to growth processes (e.g., leaf loss and flushing) could deplete current N storage in living biomass, altering the seasonality of N use within the ecosystem and reducing growth or altering phenology in subsequent years. Similar impacts would be expected on stored non-structural carbohydrate pools; reductions in storage do in fact generally occur following drought events (Hanson & Weltzin 2000). However, positive impacts of high temperatures on nutrient cycling have also been observed: Rustad et al. (2001) conducted a meta-analysis of experimental sustained warming and found that in general 2-9 years of 0.3-6 °C warming increased N mineralization by 30-64 % (95% confidence limits). However, this result cannot be generalized to acute heat shocks, and the effects of short-term high temperatures on N mineralization rates remain unknown.
1.4.2 Productivity

In addition to impacting nutrient cycling, high temperature events can have a dramatic impact on the productivity of forest ecosystems. In general, high temperatures cause a decrease in photosynthetic rates (Wahid et al. 2007); this is in part because the optimum range for photosynthetic activity tends to be moderate (15-25 °C) (Boisvenue & Running 2006). Of course, productivity is not dependent on temperature alone, but along with radiation and water it is a primary abiotic control at the global scale (Boisvenue & Running 2006). Secondary processes dynamically mediate the effects of these three main drivers; for example, temperature is also a key regulatory control on soil respiration (Norby et al. 2007). Forest productivity response to temperature is of critical interest to modern scientists and land managers because of forests' vast capacity for carbon storage. Declines in productivity, dieback and increased rates of carbohydrate consumption in cellular metabolism under extreme temperatures threaten to turn global forests into carbon sources (Allen 2009; Allen et al. 2010) with strong negative repercussions for global warming. Some work suggests that higher biodiversity will help ameliorate this response, but the existing evidence is contradictory (Archaux & Wolters 2006).

In general, extreme high temperatures negatively impact productivity, especially when coupled with drought. This is contrary to the effect of moderate warming; Rustad et al. (2001) conducted a meta-analysis and found that plant productivity increased 15-23% (95% confidence intervals) with 2-9 years of warming in the range of 0.3-6.0 °C. Under high temperatures it is argued that decreases in photosynthesis coupled with increasing dark- and photo-respiration rates will reduce productivity at the ecosystem scale (Wahid
et al. 2007); when temperature stress is combined with drought this decline has been observed to be relatively uniform despite site characteristics, ecosystem adaptive capacity, and severity of the climatic event (Ciais et al. 2005). Low productivity may persist for several years due to ecosystem adaptation to a heat-stressed environment, such as decreased LAI (Breda et al. 2006). Declines may be dramatic. Gratani (2000) observed a reduction in productivity to 50% of it's optimal level in Quercus ilex when temperatures exceeded 35.7 °C. This effect was attributed to stomatal control on evapotranspiration. It is noteworthy that productivity declines were so large despite Q. ilex being a relatively heat-adapted tree; it naturally occurs in a Mediterranean climate, and lethal temperatures for this species are in excess of 50 °C (García-Plazaola et al. 2008). Interaction effects between high temperatures and elevated CO₂ remain unclear. Bassow et al. (1994) found one day exposure to high heat (45 °C) decreased biomass for Betula spp. and Acer pensylvanicum for seedlings grown in both ambient (350ppm) and elevated (700ppm) CO₂, but that biomass losses were higher for Maples in the short term in the elevated treatment. Again, this is a research area likely to receive more attention in the future.

1.4.3 The 2003 European Heat Wave

To date, the only well-studied example of the effects of a particular, large-scale high temperature event on forests is the European heat wave of 2003. The existence in Europe of widespread forest monitoring networks prior to this event allowed researchers to assess the impacts of heat across a range of spatial scales, from controlled field experiments to regional assessments of productivity. However, in this case the effects of high temperatures and drought were severely conflated: a 6-month drought caused a soil
moisture deficit throughout the growing season, the effects of which were exacerbated by high daytime temperatures in early June and August (Roualt et al. 2006; Breda et al. 2006). Europe-wide temperature increases of up to 5 °C (averaged over the summer season) were observed, but the increase was sustained rather than episodic (Rennenberg et al. 2006). The extreme climatic conditions caused a decrease in gross primary productivity of ~ 30%, causing Europe to become a net source of CO₂, emitting ~0.5 Pg C and reversing the effects of four years of C sequestration (Ciais et al. 2005). In Western Europe this effect was driven more by high temperatures than rainfall deficit (ibid). Other authors corroborate this dramatic reduction in growth (e.g. Martinez-Meier et al. 2008).

Despite general productivity declines forest species exhibited differential plasticity in photo-oxidative stress response and acclimation, with Mediterranean species exhibiting higher plasticity than Atlantic ones (Archaux & Wolters 2006). Visual symptoms of heat damage included leaf discoloration, rolling and leaf fall; observed abscised leaves were both green and "brown [as] if oven-dried as a result of excess heat" (Breda et al. 2006: 637). Despite high temperatures and drought being tightly correlated with tree mortality no mechanistic explanation for mortality has yet been proposed (Breda et al. 2006). Interestingly, results from a free air carbon enrichment (FACE) experiment affected by the heat wave support the idea that elevated CO₂ levels (530 ppm in this study) may not alter tree response to high temperatures: 2003 stem basal area growth in trees under FACE was ~75% of previous years. Such results highlight the importance of ongoing in situ studies of forest environments: while experimental conditions may be disrupted by stochastic climatic events, this disruption provides a rare opportunity to assess the impacts of non-replicable conditions. Note, however, that while
data from the 2003 European heat wave provides a fascinating case study of the impact of high temperatures on temperate forest ecosystems, climatic conditions were fundamentally different (sustained; coincident with a severe precipitation deficit) from those observed in Ontario in May 2010.

1.4.4 Future Research Areas

While the molecular mechanisms by which extreme heat affects plants are fairly well known, almost no work investigates the impacts of acute high temperature events on mature canopy trees or at larger spatial scales. Inferences may be drawn from warming and drought studies but targeted work on the subject is very rare. Even the impacts of relatively well-studied phenomena such as droughts are poorly understood with respect to forest biodiversity and function (Archaux & Wolters 2006), and the fundamental mechanisms underlying tree responses are in many cases not known (Allen et al. 2010). Where high temperatures are considered, it is generally only insofar as they induce drought in water-limited areas (e.g. see a comprehensive meta-analysis by Lindner et al. 2010). This may be in part due to a near-total lack of information on the interactions between forest phenology and high temperature events, in which temperature stress alone may have significant impacts in the absence of water stress. There is some awareness of the importance of the timing of extreme temperature events (Bassow et al. 1994; Hanson & Weltzin 2000; Ciais et al. 2005) and the increased vulnerability to drought of young and old trees has been demonstrated (Archaux & Wolters 2006) but comprehensive studies of the interaction of phenological stage with climatic events remain an area ripe for exploration.
1.5 Ontogenetic Mediation of Damage and Recovery

Understanding ontogenetic variation in tree stress response is critical for assessing the impacts of a stress factor across all spatial scales. The impact of ontogeny on the mediation of damage and recovery in forest trees has not yet been well studied; for the specific case of high temperature stress, this author knows of no works focused specifically on the topic. What studies do consider ontogeny are mostly concerned with tree response to pests and pathogens, or resprouting following mechanical damage. Even here data are scarce, and little attention has been paid to such critical topics as the role of ontogeny in tree compensatory response to herbivore damage (Boege 2005) or resprouting in temperate trees (del Tredici 2001). Reviews do exist addressing ontogenetic variation in tolerance and recovery of herbivore damage (Boege & Marquis 2005), and the implications of leaf ontogenetic stage for pest and pathogen damage (Coleman 1986), but these draw on limited and often inconclusive data.

Dendrochronological studies examining ontogenetic variation in climate sensitivity (section 1.3) are also a useful source of information, but as yet have not found a universal trend in age response to warming.

Fortunately, the highly successful (Weiner 2004) concept of allocation, and resulting econometric analyses, have motivated several authors to offer theoretical explanations for ontogenetic changes in plant damage tolerance and post-damage recovery that can be generalized to the specific case of the impact of high temperature stress on forest trees. Furthermore, the results of some authors (e.g. Wen et al. 2008; Reyes-Díaz et al. 2009) indicate that ontogenetic mediation may be broadly related to life
history strategy and functional group class, and where this information is available for a species it may have weak predictive power. Thus although no targeted theoretical explanation exists, allocation theory and known age- and size-related physiological changes provide useful theoretical frameworks for understanding ontogenetic variation in tree damage and recovery.

### 1.5.1 The Principle of Allocation

The idea of allocation is simple, powerful and familiar: plants exist in a condition of limited resources, and must allocate them between competing demands (See Bazzaz et al. 1987 for a review of the principle of allocation). Trade-offs between demands are implicit, leading to the development of life-history strategies (Weiner 2004) in which ontogenetic heterogeneity in selective pressures, shifting demographic priorities and allometric scaling cause plants to prioritize different functions at different ontogenetic stages (Boege & Marquis 2005). When examining allocation it is important to distinguish between changing functional priorities and increases in plant size. Together these represent the main ontogenetic drivers of changing resource allocation (Boege & Marquis 2005), but size-related changes are, properly speaking, allometric rather than allocational. Because plant growth itself is allometric, constant ratios of allocation between competing demands may appear to vary throughout ontogeny, a phenomenon termed 'apparent plasticity' (Weiner 2004).

The principle of allocation predicts trade-offs between allocation of resources to reproduction, growth and defense (Bazzaz et al. 1987); these changes in allocation are a fundamental aspect of a genotype's life history strategy (Weiner 2004). It has been clearly
shown that trees allocate increasing resources to reproduction with age (Thomas 1996), beyond a minimum threshold size (Weiner 2004). Multiple papers from the Thomas Lab at the University of Toronto have explored how increased investment in reproduction may be responsible for ontogenetic shifts in tree functioning. Nock et al. (2008) found declines in intra-crown leaf area index with increasing age, and hypothesized that increased investment in reproduction as well as ontogenetic changes in light competition strategies could be responsible. Thomas et al. (2010) examined ontogenetic patterns of herbivory in Sugar Maple and suggested that reproductive N demands may decrease leaf palatability late in tree ontogeny, an interesting example of a trade-off between reproduction and defense that may not be zero-sum. Recently, a comprehensive study (Thomas 2010) found that photosynthetic capacity (assessed as Amax\_area, Amax\_mass and related parameters such as stomatal conductance) peaks prior to reproductive onset in three temperate deciduous trees including Sugar Maple, providing strong support for the notion that increased allocation to reproduction motivated by life-history strategy may be responsible for ontogenetic declines in other aspects of tree function.

The principle of allocation has also been applied to study ontogenetic variation in plant stress response, notably plant defense and tolerance to herbivory and tradeoffs in resprouting and re-seeding responses following damage. A recent review (Boege & Marquis 2005) on plant allocation to defense and tolerance throughout ontogeny has argued that defense/tolerance will be lowest in seedling stages and when plants are senescing. Using a resource based econometric approach, the authors evaluate the competing demands of growth and defense/tolerance throughout an idealized plant life cycle, arguing that germinating plants (the "cotyledon stage") can draw on stored
resources and so can achieve moderate levels of defense/tolerance. As a seedling develops, relatively more resources must be allocated to growth, reducing defense/tolerance. Development into a sapling slows the rate of growth relative to resource uptake, allowing more investment in defense/tolerance. Following reproductive onset it is suggested that allocation to reproduction will eventually cause investment in defense/tolerance to decline to nearly zero when trees are senescent. This last point is contentious. It has been suggested (Loehle 1988) that defensive investment may determine longevity, implying that longer-lived species do not decrease their investment in defense/tolerance until close to senescence and death. Recent work (Thomas 2010) has found that leaf chemical and morphological traits associated with investment in defense increase linearly with tree age in three temperate species (including sugar maple) invalidating the idea that defensive investment decreases with tree age as a general rule in mature trees.

Despite proposing a generalized model based on the principle of allocation, Boege & Marquis (2005) find that studies examining ontogeny-defense relationships have yielded mixed results. There are several possible explanations. Older Sugar Maples have been shown to suffer from increased herbivory (galling arthropods) (Thomas et al. 2010), and increased pest or pathogen loads in older trees could give the appearance of declining defensive investment. Most studies compare only two ontogenetic stages (Boege & Marquis 2005), a research approach that can overlook or misrepresent true ontogenetic trends (e.g. Thomas 2010). Lastly, the lack of coherence among studies examining ontogeny-defense relationships may be attributable to interspecific differences in life history strategies or in demographic selective pressures. For example, work with Betula
pendula Roth. and B. pubescens Ehrh. suggests that birch seedlings may be at a lower risk of attack (have lower "apparency" to herbivores) than mature trees (Fowler 1984), suggesting a selective pressure against seedling defensive investment that may not be present in other trees.

Investment in defense and tolerance represents a form of damage avoidance, which in turn can be viewed as a resource allocation option (avoidance) that trades off with recovery ability (Vesk 2006). Bellingham and Sparrow (2000) present a general explanation for resprouting versus reseeding in plants, arguing that disturbance severity and nutrient availability determine whether a damaged plant reseeds (intermediate damage; few resources available for repair) or resprouts (mild or extreme damage; resources available). Since resource availability is generally related to plant age/size, the relative magnitude of damage : resources may be expected to produce ontogenetic variation in plant response to a discrete damage unit. For example, the authors find that when most above ground biomass is lost older plants have larger stored resource pools and can resprout, while for younger plants the ratio of damage : resources is catastrophic.

1.5.2 Allocation, Physiology and Ontogeny

The specific developments of allocation theory outlined in section 1.5.1 are informative for an analysis of the effect of high temperature stress on forest trees, but as noted no targeted work exists. It may therefore be useful to consider how an allocational explanation can inform observed physiological traits associated with aging that may also underlie ontogenetic mediation of damage and recovery. Increases in the carbon/nutrient balance, plant storage capacity and access to water and nutrients, as well as decreases in
the root:shoot ratio and growth rate (Boege 2005) alter the allocation of resources within aging plants, generally making more resources (carbon, nutrients and water) available for older trees to use in damage avoidance and recovery. However, older trees also undergo changes associated with senescence such as decreased photosynthetic rates, stomatal conductance, hormone production, decreased metabolic rates and slower wound healing (Kozlowski 1969; Boege 2005). These changes are not well explained by allocation theory, and may confuse attempts to explore physiological changes using the concept. Thus while older trees generally have access to more resources and have reduced allocation of resources to growth, tending to increase damage compensation ability (Boege 2005), allocation-independent physiological changes reduce damage tolerance and recovery capacity in older trees. Intermediate and mature stages, having a strong 'allocative position' and not yet senescent, may be the most resilient. This conclusion is borne out by field data, with intermediate ontogenetic stages shown to have the greatest acclimation response following hurricanes (Wen et al. 2008) as well as high photoprotective (Reyes-Díaz et al. 2009) and photosynthetic (Thomas 2010) capacity, and water-use efficiency (Cavender-Bares & F.A. Bazzaz 2000- but WUE (here viewed as the ratio of CO₂ assimilation to water consumed) has also been found to increase monotonically with tree size; see Thomas 2010). However, note that these studies (with the exception of Thomas 2010) suffer from not considering the full range of ontogenetic stages, generally focusing on only two (seedlings vs. mature trees).
1.5.3 **Size-dependent Access to Resources**

Since allocation theory requires damage tolerance or recovery to be constrained by a condition of limited resources, characteristic differences in ecosystem resource share between ontogenetic stages can be expected to partly define the response of age/size cohorts. Differences in resource access between small and large plants could influence trends in ontogenetic responses to abiotic stress. It is generally accepted that plant resource share scales with plant size and dominance (Silvertown & Dale 1991; Binkley et al. 2002; Boege 2005), but this scaling is generally not symmetric throughout ontogeny. Larger plants tend to have a disproportionate share of available resources, a phenomenon known size asymmetry in resource competition (Weiner & Thomas 1986; Thomas & Weiner 1989; Bengtsson et al. 1994; Schwinning & Weiner 1998). It has been argued that whether competition for a resource is size symmetric or asymmetric depends on the excludability of the resource (Schwinning & Weiner 1998), that is, whether use by one plant pre-empts use by another. Due to directionality in supply, light is the most obvious example of an excludable resource.

Despite numerous predictions little is known about size asymmetry in water use and soil resource use (Schwinning & Weiner 1998), but it has been argued that below-ground resource competition is more symmetric than aboveground due to heterogeneity in soil resource distribution and high redundancy in root architecture, both of which make it harder for large plants to dominate access to below-ground resources (Casper & Jackson 1997; Schwinning & Weiner 1998). Size asymmetry in water resource use remains similarly unexplored, but it is known that ontogenetic changes in rooting depth give older trees access to deeper water sources (Weltzin & McPherson 1997), clearly
beneficial in times of water scarcity. It has also been suggested that ontogenetic changes in whole-plant water potential and stomatal regulation could drive size asymmetry in competition for water, but this hypothesis has not yet been tested (Schwinning & Weiner 1998). Lastly, note that ontogenetic variation in resource share may be conflated with differences in efficiency of resource availability, depending on experimental design. No consensus has yet emerged as to the relationship between ontogenetic stage and efficiency of whole-plant resource use, although many studies suggest (often contradictory) trends (Binkley 2004).

### 1.5.4 Resprouting

Studies assessing resprouting following mechanical damage are highly applicable to the case of heat stress response, as resprouting represents a general compensatory pathway clearly relatable to allocation theory. Rough ontogenetic patterns in stump resprouting capacity in temperate woody angiosperms have been summarized by del Tredici (2001): most temperate woody angiosperms exhibit vigorous stump sprouting at smaller sizes (dbh 5-15cm) which is maintained at intermediate sizes (25-30 cm) but declines towards zero past ~30 cm dbh. However, stump sprouting represents only one kind of resprouting response, and may be constrained by decreased vascular connectivity and increased risk of infection in larger trees (del Tredici 2001). However, compensatory response in leaf resprouting (reflushing) in a tropical tree subjected to three levels of experimental defoliation (0, 25% and 75% of total leaf area) has also been shown to be greatest in saplings (Boege 2005). Both saplings and mature trees in this experiment were able to compensate for 25% defoliation, but when more leaf area was removed saplings
showed an increased compensatory response while mature trees did not; the author suggested decreased mobility of resources within individual trees and increasing investment in defense and reproduction as possible causes. These results suggest younger trees may in general exhibit a more vigorous resprouting response than older trees.

However, it is clear that interspecific variation exists in ontogenetic response. The coincident spatial occurrence of ice storms and Sugar Maple have provided some data regarding recovery from damage in the species—although, unfortunately, no study has examined the influence of ontogeny. Along with several other species, Sugar Maple is classed as 'most likely' to resprout following crown damage among common eastern North American trees (Brommit et al. 2004); the authors found no correlation between severity of crown damage and number of resprouting shoots, but did not relate either of these variables to tree age or size. However, resprouting may be in part explained by site characteristics, as other authors have assessed the resprouting capacity of Sugar Maple as intermediate (Duguay et al. 2001). Ontogenetic variation has not been explicitly considered but may be inferred, as younger seedlings have been found to have lower and mature trees higher than average resprouting capacity compared to other common North American hardwoods (Duguay et al. 2001); other work (del Tredici 2001) has assessed the resprouting capacity of Sugar Maple seedlings as among the lowest of forest species from this region. Within the framework of allocation theory these findings may be related to the life history strategy of Sugar Maple, with highly shade-tolerant slow growing seedlings having fewer resources to invest in resprouting than older, more vigorous, resource-dominant mature trees.
1.5.5 Applicability of Reviewed Studies

As has been seen, relatively little is known about potential ontogenetic mediation of damage and recovery in forest trees. Allocation theory provides a general framework within which to assess the implications of changing life-history priorities between ontogenetic stages, but any attempt to generalize these ontogenetic changes is complicated by interspecific variation, site-specific differences, and the different characteristics of various damage types. Assessing the implications of ontogeny for response to extreme high temperature events involves a specific set of physiological pathways that may be quite different from those involved in response to insect herbivory (for example). Thus studies into age/size mediation of damage in forest trees following hurricanes have concluded that larger individuals are generally more severely impacted (Everham & Brokaw 1996; Paciorek et al. 2000), while recovery and survivorship following fire increases with size and age (Vesk 2006). The damage pattern is quite different in both cases, and scales differently with ontogenetic stage. Available data do indicate that Sugar Maple has a high capacity for resprouting, and that resprouting response may vary ontogenetically, but ontogenetic variation in response to heat stress remains unexplored.

1.6 Vertical Gradients in Tree Canopies

Tree crowns, and thus forest canopies, often appear to be relatively homogenous. Easily observable characteristics such as leaf density or color may not appear to vary from the bottom to the top of the crown, and in fact even highly refined quantitative disciplines such as ecosystem productivity modeling have often treated conditions within
canopies as static versus height (so-called "big leaf" models). In reality the situation is quite different: an increasingly large body of work has revealed that abiotic gradients within canopies, including but not limited to light availability, cause large structural and functional modifications to foliage (Niinemets 2007). The consequences of this variation are large, increasing photosynthetic capacity from 2-4 times from the bottom to the top of the canopy (Niinemets 2007) and locating up to 40% of daily carbon assimilation in the uppermost 10% of the crown (Caldwell et al. 1986). Studies have indicated that these differences are mostly the result of structural rather than biochemical variation in leaves along a vertical gradient (e.g. Ellsworth & Reich 1993), although biochemical alterations clearly play a significant role (e.g. Leal & Thomas 2003)). However, despite numerous studies and several excellent reviews (e.g. Niinemets & Valladares 2004; Hikosaka 2005; Niinemets 2007) on the subject of vertical gradients in form and function in tree canopies, important gaps in our understanding remain. In particular, while within-crown variation in leaf form and function has been studied (e.g. Jones & Thomas 2007)), little is known about vertical trends in damage and recovery.

Section 1.6 aims to provide a brief review of known trends in foliar form and function across vertical gradients in tree canopies and relevant ecophysiological theory. Note that alterations to crown architecture (branching, form and extension) and hydraulic conductivity will not be independently considered. Changes in leaf characteristics are i) most germane to the present work, ii) sufficiently well studied to merit review, and iii) convey implicit information on crown architecture and conductivity sufficient to summarize these related topics as they pertain to an investigation into productivity, leaf response to heat, and reflushing.
1.6.1 Abiotic Gradients

Observed height related trends in abiotic factors within canopies are the result of a dynamic interaction between fluctuating environmental conditions and current vegetation cover. Foremost among these is the reduction in light with increasing canopy depth, which may span several orders of magnitude (Niinemets & Valladares 2004). Irradiance reaching the forest floor is typically 2% of light reaching the top of the canopy (Naidu & DeLucia 1997), but this value varies with species and age. In an even-aged mature Sugar Maple-dominated hardwood stand this proportion has been found to be slightly less than 2% (Ellsworth & Reich 1993), but a recent study (Nock et al. 2008) showed that ontogenetic differences in intra-crown LAI ("crown thinning") cause light penetration increase exponentially with tree size in Sugar Maple (<1% at 20 cm dbh to 4% at 70 cm dbh, predicted to reach 8% at ~90 cm dbh). Light transmission in Betula alleghaniensis was also examined in this study and did not exhibit as extreme an increase (<2% at 70 cm dbh), demonstrating interspecies differences in ontogenetic variation in light transmission. Water vapor pressure deficit scales positively with light, as does temperature (Niinemets 2007), which is typically reduced by 20-30% in the bottom of the canopy and can create disparities of up to 10 °C (Niinemets & Valladares 2004). Similarly, wind speed and mechanical stress on leaves increase with increasing canopy height (Niinemets & Valladares 2004). Resultant gradients in crown damage have been linked with strong changes in foliar morphology and chemistry (Leal & Thomas 2003).

In general, then, vertical gradients in abiotic factors within forest canopies represent a movement from bright, more extreme conditions closely linked to
environmental conditions at the canopy top into an increasingly darker, less linked forest understory. Given that much work focuses on acclimation of leaves at different canopy levels to abiotic gradients, it is interesting to consider that these same gradients are at the same time created by the growth of the tree. Thus heterogeneity in leaf size, distribution and orientation determines light availability within the canopy (Ellsworth & Reich 1993), and changes in cumulative leaf area index (LAI) above any given canopy point affect mass and energy exchange, turbulence and ambient gas concentrations (Baldocchi et al. 2002). It is this understanding that has led many authors to consider trends in leaf physiology and morphology within the crown as an attempt to optimize resource use rather than a simple response to acclimation. The 'abiotic acclimation' perspective is further complicated by variations in function and by stress within the crown: statistical partitioning of the effects of light environment from vertical height has indicated that factors other than light acclimation (e.g, mechanical damage, reproductive investment) influence vertical gradients within the crown (Leal & Thomas 2003).

1.6.2 Leaf Structural and Functional Responses

Whole-plant maximization of photosynthesis along abiotic canopy gradients alters shoot architecture, gross leaf morphology, internal leaf architecture and leaf chemistry on a per unit area basis. Shoot architectural changes tend to structurally maximize the area of photosynthetic tissue receiving light. Upper canopy leaves are thicker, more clumped, and oriented close to the vertical, while lower canopy leaves are thinner and oriented close to horizontal (Baldocchi et al. 2002; Niinemets 2007). Increasingly acute leaf angles and clumping in the upper canopy can be thought of as
'shoot packing', or a shoot architectural structure that maximizes total leaf area. Leaf angles in Sugar Maple have been found to exhibit significant but very slight variation between the bottom and the top of the crown; no significant variation was found with dbh (Nock et al. 2008). Although leaves at angles close to the vertical only harvest light with (typically) only 15-20% efficiency relative to a flat leaf, light in the upper canopy is generally much higher than needed to saturate leaf photosynthesis (Niinemets 2007). Shoot packing also increases light penetration to the lower canopy (Niinemets 2007), and provides a structural means by which leaves can avoid photoinhibition associated with excess light absorption (Niinemets & Valladares 2004). Similarly, in the lower canopy flatter leaves (more obtuse leaf angles) maximize light harvesting efficiency (Niinemets 2007). It is known that within-canopy shading affects leaf morphology (Poorter et al. 2006) and general trends in shoot morphology may thus also be associated with horizontal crown depth. Interestingly, vertical changes in individual leaf structure exert only a moderate influence on leaf light absorbance, except when leaf surfaces become extremely waxy or hairy (Niinemets 2007).

1.6.3 Leaf Size, Weight and Thickness

Changes in leaf gross morphology are best explained by variation in leaf thickness and thinness. Leaf mass per area (LMA) varies (positively) with light, with leaves in more shaded portions of the canopy exhibiting lower LMA (Ellsworth & Reich 1993; Poorter et al. 2006). Variation in LMA reflects integrated light levels rather than peak irradiance (Ellsworth & Reich 1992), and variation increases with height (light) (Baldocchi et al. 2002). Increases in LMA are due to thicker mesophyll, which is highly
correlated to leaf photosynthetic capacity (Jurik 1986), and thus variation in LMA may be more important than leaf nitrogen content in the acclimation of leaves to different light environments (Evans & Poorter 2001). Height/light-related changes in LMA are dramatic and probably universal across taxa and functional groups (Poorter et al. 2006); in Sugar Maple LMA values of ~0.004 g cm\(^{-2}\) (canopy bottom) to 0.007-0.0010 g cm\(^{-2}\) (canopy top) have been reported (Jones & Thomas 2007). This is consistent with Jurik's (1986) finding that LMA decreases exponentially with increased shading in northern hardwood species. In this study (Jurik 1986) Sugar Maple was found to have a low average SLW among other deciduous hardwoods; this is consistent with the tree's observed shade tolerance (Farrar 1995), indicating that in general its leaves are adapted to lower light environments. Finally, thinner leaves (lower LMA) in the lower canopy also tend to be larger, probably to increase interception of scarce light (Poorter et al. 2006).

### 1.6.4 Leaf Internal Architecture

In addition to changes in shoot packing and gross leaf morphology, altered leaf internal architecture in response to changes in leaf light environment and height has also been shown to affect leaf function. Within-leaf CO\(_2\) conductance is considered to limit assimilation as strongly as stomatal conductance, and is related to the porosity of the mesophyll layer (Oguchi et al. 2005). While positive correlations have been found between internal conductance and anatomy the relationship has been shown to be weak in three *Acer* species (Hanba et al. 2002), not including *A. saccharum*. The size and arrangement of mesophyll cells also determines the quantity of chlorophyll that can be located with access both to light and to gas diffusion in and out of the leaf. Plants grown
under high irradiance have been shown to have larger mesophyll cells with more sites suitable for chlorophyll than low-light controls (Oguchi et al. 2005), and mesophyll area has been shown to increase with height in *Pinus strobus* (Leal & Thomas 2003). Upper canopy leaves are also generally more rigid and have lower leaf osmotic potentials than lower canopy leaves, which may improve their ability to draw water from the soil (Niinemets & Valladares 2004).

### 1.6.5 Chlorophyll and Nitrogen

Leaf chlorophyll and N content both vary with canopy light environment, and have been shown to vary with height independent of light in *Pinus strobus* (Leal & Thomas 2003). It is important not to conflate this variation with trends in gross leaf morphology (section 1.6.3). It is useful in this regard to distinguish between chlorophyll and nitrogen concentrations per unit *area* and per unit *mass*. Increasing LMA with canopy height will lead to apparent variation in per unit area measurements despite comparable leaf biochemistry across canopy heights, while disparities between both measurements can be used to explore how structural responses facilitate leaf acclimation to light environments (e.g. Ellsworth & Reich 1993; Evans & Poorter 2001)).

Measurements per unit (dry) mass are most useful for isolating the biochemical components of photosynthetic acclimation. Chlorophyll content per dry mass typically increases with decreasing light availability in the canopy, reflecting both increased investment in photosynthesis and the more efficient use of structural elements in a condition of increasing resource scarcity (Niinemets 2007). Relationships have been established to extrapolate chlorophyll content from % leaf N (Niinemets 2007), and it has
been argued that leaf N concentration exhibits a general linear relationship with photosynthetic capacity due to large N investments in Rubisco (Meir et al. 2002). Ellsworth and Reich (1993) found a linear relationship ($A_{\text{max}}/\text{area}=0.847 + 5.026*N/\text{area}$) for Sugar Maple, but recent work by Jones and Thomas (2007) with a much larger sample size (12 trees vs 3 trees in the Ellsworth and Reich study) invalidated this argument, finding a strongly non-linear relationship.

On a per area basis canopy N has been shown to vary significantly across canopy microsites (Hollinger 1996), with the brightest sites having the highest N concentrations. As with LMA, variation in N per unit area (and the related measure, maximum carboxylation capacity $V_{c_{\text{max}}}$) has been found to increase with height / brightness (Baldocchi et al. 2002). Evans and Poorter (2001) observed changes in leaf N per area and attributed them to gross morphological variation (LMA); the authors argue that LMA increased rather than N concentration because of internal anatomical limits to within-leaf $\text{CO}_2$ diffusion. On a per mass basis this study found no significant variation between high light and low light treatments, but low light plants partitioned a larger N fraction into light harvesting than high light plants (21% vs 13% on average). This is consistent with the arguments of Niinemets (2007) reported above. While it has been suggested that constant N per unit mass and structurally driven variation in N per unit area are general trends (Evans & Poorter 2001), more recent work (Leal & Thomas 2003) found decreasing N per unit mass with increasing height. The lack of coherence among results of studies examining vertical gradients in N per unit mass could be the result of interspecific differences in LMA gradients throughout the canopy (Leal & Thomas 2003). Leaf age may also affect N content, the two exhibiting a negative correlation (Field
and several studies have shown that leaf aging sequence along with variation in microhabitat and canopy light gradient may influence canopy variation in leaf N per area in Sugar Maple (see Ellsworth & Reich 1993). Thus while structurally driven increases in N with light on a per area basis are well established, trends in N per mass exhibit interspecies variation and may not be general.

1.6.6 Photosynthetic Capacity

Given substantial variation in gross leaf morphology and leaf nitrogen and chlorophyll concentrations it is unsurprising that leaf photosynthetic capacity has also been observed to vary, and that this variation has generally been observed on a per unit area basis. Photosynthetic capacity per unit leaf mass for upper versus lower canopy or for high versus low light treatment leaves has been shown to be not significantly different across a variety of plant systems including Sugar Maple (Ellsworth & Reich 1992; Ellsworth & Reich 1993; Evans & Poorter 2001; Poorter et al. 2006), but the results of Jones and Thomas (2007) contradict this generalization. These authors found that $A_{\text{max}}$ (on a per mass basis) was significantly different between upper and lower canopy positions; vertical trends in reproductive N allocation and the effects of crown damage (Leal & Thomas 2003) as well as changes in physiological factors such as hydraulic conductance could account for this discrepancy.

On a per unit area basis, photosynthetic capacity (e.g., $A_{\text{max}}$) scales positively with light gradients in tree canopies (Poorter et al. 2006). $A_{\text{max}}$ per area in Sugar Maple has been found to vary (inter-annually across three years) from $\sim$4-6 µmol m$^{-2}$ s$^{-1}$ (bottom canopy) to $\sim$6.5-9 µmol m$^{-2}$ s$^{-1}$ (Jones & Thomas 2007); work in a Sugar Maple-
dominated mixed hardwood forest correlated a similarly large increase (5.2 µmol m⁻² s⁻¹ in the lower canopy versus 10.9 µmol m⁻² s⁻¹ in the upper) to cumulative LAI above the sampled leaf (Ellsworth & Reich 1992). A comparable decrease of roughly 2x was found between high and low light treatment leaves in 10 C₃ dicot species representing a range of growth forms and functional groups (Evans & Poorter 2001). Because changes in leaf morphology (notably LMA) may partially drive height-related trends in per area photosynthetic capacity (Ellsworth & Reich 1993; Evans & Poorter 2001; Niinemets 2007), morphological parameters such as LMA may be effective tools to integrate the influences of canopy structure and light environment on leaf photosynthetic performance (Ellsworth & Reich 1993).

However, despite the focus on structural explanations for variations in $A_{\text{max}}$ on a per area basis, some biochemical variation evidently does occur. Upper canopy leaves exhibit larger xanthophyll pool sizes and enhanced non-photochemical relative to lower canopy leaves, and the optimum temperature for photosynthesis increases with light throughout the canopy (Niinemets & Valladares 2004). This suggests that upper canopy leaves will be more tolerant of heat stress, and lower canopy leaves suddenly exposed to high temperatures will likely do badly. As a general trend more shade tolerant species can be expected to be more susceptible to photoinhibition at all canopy levels (Naidu & DeLucia 1997), but is unclear how the interacting effects of temperature and VPD will affect photoinhibitory response across canopy gradients (Niinemets 2007). Finally, tradeoffs can exist between $A_{\text{max}}$ and light harvesting efficiency (Niinemets 2007), with more shaded leaves generally exhibiting greater metabolic efficiency (Ellsworth & Reich 1993).
1.6.7 Optima and Deviations

The observation that the trends in leaf form and function described above are the result of vegetation growth interacting with directionality in resource availability has motivated a number of studies which regard observed canopy gradients as a means of whole-plant photosynthetic optimization. Drawing on econometric and evolutionary theory, it is predicted that N allocation should change with light extinction within a canopy so as to maximize whole-plant photosynthesis (Ellsworth & Reich 1993); this idea has become known as the 'acclimation hypothesis' (Meir et al. 2002). Given that this idea explicitly builds on evolutionary theory it is interesting that no work to date has integrated optimization throughout plant life-span, or throughout specific life-history stages (e.g. establishment, understory competition, maturity, senescence). Current work evaluates canopy N distribution as a proxy of photosynthetic investment and capacity, and compares observed trends against theoretical optima derived from mechanistic models (e.g. Hollinger 1996; Meir et al. 2002; Hikosaka 2005); results indicate that a tight positive correlation between canopy N distribution and light availability maximizes photosynthesis (Meir et al. 2002). This conclusion is highly coherent with observed trends in canopy N and photosynthesis (reviewed above), and has been experimentally supported. For example, Hollinger (1996) found that the actual distribution of N throughout a tree canopy produced whole-canopy photosynthetic rates higher than 99% of random combinations of foliage characteristics and canopy levels.

Optimization can also been seen through an econometric lens, placing the emphasis on light compensation points below which the costs of leaf construction or
increased N concentrations are not justified by assimilative returns (Hikosaka 2005). In this vein low-light leaves have been shown to have 'payback times' 2-3x greater than high-light leaves across multiple dissimilar plant systems, including forest canopies (Poorter et al. 2006). An alternative approach by Chen et al. (1993), termed coordination theory, attempts to link optimization more closely with mechanistic models by considering N allocation as an attempt to balance Rubisco limitation and electron transport limitation on carboxylation in light of both processes' competing N demands. However, this attempt to re-focus optimization at the biochemical rather than whole-plant level remains relatively unexplored and observed (ubiquitous) deviations in N distribution and photosynthetic rates from theoretical optima have yet to be accounted for (Niinemets 2007). The current best explanations are carbon 'costs' to N allocation such as leaf-building (Hollinger 1996), interacting stresses such as wind, herbivory, and photoinhibition (Hollinger 1996; Niinemets & Valladares 2004), and evolutionary tradeoffs reducing adaptive plasticity within tree canopies (Niinemets & Valladares 2004).

1.6.8 Plasticity and Adjustment

Since tree canopies are dynamic rather than static systems, within-canopy acclimation to abiotic gradients is continuously modified by leaf-level adjustment to changing micro-environmental conditions. Both structural and biochemical plasticity determine leaf responses to changed conditions. While leaves are highly plastic to their growth conditions, tending to acclimate to their local light regime (Jurik 1986; Naidu & DeLucia 1997; Meir et al. 2002), plasticity decreases with increasing leaf age. Niinemets
(2007) reviews (in part) the literature pertaining to leaf adjustment and finds that constraints are mostly structural; adaptation in shade-developed leaves may also be influenced by N availability (Naidu & DeLucia 1997). In the short-term adaptation to increased light can be achieved by rapid fluctuations in xanthophyll pool size (Niinemets et al. 1998), although the role of such short-term adjustment in long-term adaptation is not yet clear (Niinemets 2007).

It is perhaps more useful to consider only semi-permanent changes as adaptation, and remove highly reversible changes to dynamic biochemical systems from the discussion. While developing leaves can rapidly adapt to an altered light environment, cross-linked and lignified cell walls in mature leaves prevent increases in LMA, and restrict adaptive options to increasing chlorophyll concentration and the availability of limiting biochemical components (e.g. increased N investment in Rubisco). This emphasis on structural limits to adaptation was experimentally supported by the work of Oguchi et al. (2005), who found that plasticity in mesophyll growth (increased surface area) and the availability of unoccupied cell surface suitable for chloroplast expansions were the main determinants of light adaptation potential in mature leaves. In this experiment $A_{\text{max}}$ was found to increase only with increasing chlorophyll volume when leaves were transferred from low to high irradiance. Interestingly, an early successional species (*Betula ermanii*) was found to produce significant vacant space on mesophyll cell surfaces in low-light leaves, facilitating rapid acclimation to high light levels (and thus maximizing gap-release response), and suggesting rough linkages between plant life history and leaf adaptive response. Finally, some work suggests that the upper canopy
may in general be more plastic to changing light environments, and may thus have more capacity for adaptation (Niinemets 2007).

As a shade-adapted tree, Sugar Maple may be relatively unable to adapt to changing light environments. This conjecture is borne out by the work of Naidu and DeLucia (1997), who examined photosynthetic response in shade-grown seedlings exposed to high light. Sugar Maple seedlings exhibited increased $A_{max}$ when transferred into high light (~13 vs ~ 8 µmol m$^{-2}$ s$^{-1}$), as well as increased LMA (3 vs. 2.4 mg cm$^{-2}$), but this acclimation was significantly less than in the less shade-tolerant species *Quercus rubra* L. Sugar Maple also sustained photoinhibition for longer than Oak, and photosynthetic rates may be limited by long term photoinhibition. A similar adaptive response, though of lesser magnitude, was found by Ellsworth and Reich (1992). In a study of mature canopy trees Jones and Thomas (2007) found that Sugar Maple leaves did not acclimate to increased light levels following gap creation for two years, by which point acclimated leaves attained rates of ~4 µmol m$^{-2}$ s$^{-1}$ versus ~7 µmol m$^{-2}$ s$^{-1}$ for the control. The authors suggest slow leaf morphological adjustments possibly resulting from predetermined palisade layers and cell numbers as a cause for this lag. Despite this work on acclimation in Sugar Maple, the biochemical portion of adaptation in the species remains to be quantified. It is known that the species has a high plasticity of N investment to light-harvesting capacity (1993) but anatomical adjustments in foliar structure are considered to play the major role (Niinemets & Tenhunen 1997). This opinion is borne out by observed changes in mesophyll thickness and gas diffusion in other *Acer* species (Hanba et al. 2002).
1.7 Sugar Maple Decline

Any discussion of possible wide-spread dieback in North American Sugar Maples will likely draw comparisons with a phenomenon known as 'maple decline', a consequence of both the large body of work on this subject and its past high visibility in Canadian media and agriculture. While it will be argued that key aspects of maple decline are highly dissimilar from the heat-shock phenomenon examined in this thesis (section 2.3.9) the subject is highly related and merits review, most critically as it represents one of few studied cases of widespread forest dieback in which high temperatures but not drought have been suggested as a cause of mortality (Allen et al. 2010). This section will draw on contemporary retrospective and historical papers to briefly introduce the major characteristics and proposed causes of maple decline.

Decline, also commonly referred to as dieback or, perhaps misleadingly, 'maple blight', first came to the attention of the scientific community in the late 1950's, following a large defoliation triggered decline in Wisconsin (Houston 1999). Widespread and severe outbreaks in Southern Quebec during the late 1970s and early 1980s prompted a flurry of papers, although investigators were not able to establish consensus as to the causes of the problem. It is noteworthy that the same period saw widespread forest decline in Europe (Payette et al. 1996), as European literature suggesting rain-borne acid deposition derived from industrial pollution as the prime agent of forest decline in Eastern Europe probably played a large role in focusing attention on the same issue in North America (e.g. Houston, 1999). However, while anthropogenic causes were widely tabled, many authors came to see decline as a natural process, and in that regard it is important that maple decline has been known in North America since at least the early
1900s (Houston 1999). Following this thinking, it has been proposed that the apparent acceleration of observed decline post-1950 is perhaps more due to increased effort on the part of the observers rather than any change to the rate of decline itself (McIlveen et al. 1986).

Adding to the confusion, it proved difficult to determine when exactly these large-scale declines had begun- a critical point for any investigator interested in causal factors. This was in part due to the large (provincial) scales over which decline occurred, local heterogeneity in onset times, and (most critically) the nature of decline itself. Episodes of sugar maple decline appear to be multi-year, exhibit an accumulation of symptoms amid declining tree health, and eventually terminate in either death or recovery (Westing 1966; Griffin 1965; Hendershot & Jones 1989). Due to the gradual onset, decline may not be recognized until it has been underway for some time. Thus Hendershot & Jones (1989) discuss maple decline in Quebec from the years 1982-1987, while a dendrochronological study by Roy et al (2004) indicates that that initial stage of dieback in this region in fact occurred from 1974-1978. What is agreed upon, however, is the severity of the problem. Maples in Quebec undergoing decline exhibited mortality rates of 10-15%/year in some regions (Hendershot & Jones 1989). Since the tree species accounts for ~ 17% of commercial forest area in the province (Duchesne et al. 2005) the economic and ecological implications are considerable, to say nothing of the temporary losses in growth and syrup production in trees that survived the episode.
1.7.1 Characteristics

Key symptoms of sugar maple decline are chlorosis and leaf scorch, premature color change, early leaf abscission, crown dieback (progressing from the exterior to the interior of the crown), reduced growth, root necrosis and in some cases the sloughing of bark from the main branches and trunk (Westing 1966; McIlveen et al. 1986; Hendershot & Jones 1989; Roy et al. 2004). Trees from a declining stand have been shown to have lower photosynthetic rates and Ca and Mg concentrations but similar concentrations of non-structural carbohydrates relative to a healthy stand (Liu et al. 1997). Several studies (e.g. Westing, 1966; Roy et al., 2004) report the presence of small, pale leaves in the top of the crown. Note that while not all authors report all symptoms the high degree of concordance suggests that variation may be due to the rate and intensity of decline (e.g. presence or absence of bark sloughing) or may be a function of observation method and thoroughness (e.g. spotting or failing to spot pale leaves high in the crown), at least in the absence of a unique symptom with clear etiology (e.g. sudden massive defoliation caused by insects).

Whether younger/smaller Sugar Maples exhibit decline symptoms on par with older/larger trees seems to be a point of contention. Griffen (1965) asserts that dieback is limited to trees with a dbh. over 6 inches (15.23 cm), and Payette et al. (1996) report that decline symptoms were restricted to canopy trees, but Houston (1999) reports on the findings of several studies in which afflicted or dying saplings constituted part of a general maple decline. The implications of ontogenetic stage of onset are obviously of some importance to both researchers and forest managers; certainly those authors advancing what may be termed the 'maturation hypothesis' (see below) imply that
younger trees are less affected or exempt. The point also has ecological consequences: enhanced seedling growth (and consequent changes in age cohort composition) has been reported where younger trees were unaffected (Payette et al. 1996). More commonly a general decline of Sugar Maple at all ages produced a shift in stand composition, generally towards increased numbers of *Fagus grandifolia* (Duchesne et al. 2005).

At the landscape scale, spatial frequency of Sugar Maple decline is patchy, with healthy areas, slightly affected, moderately affected and severely affected areas intermixed at the landscape scale (Hendershot & Jones 1989). Early occurrences of decline were thought to occur mostly along roads (Westing 1966), but later reports invalidate this idea (e.g. Drohan et al. 2002). More comprehensive reviews suggest that increased severity of decline is correlated with poorer, drought-prone soils at (relatively) high elevations (Hendershot & Jones 1989; Drohan et al. 2002).

### 1.7.2 The Multiple Stress Hypothesis

Throughout the latter half of the twentieth century a number of explanations have been suggested to account for the decline phenomenon, ranging from toxic concentrations of roadside de-icing salt (Westing 1966) to anthropogenic climate change (Auclair et al. 1996). Early investigators (e.g. Westing 1966; Griffin 1965) recognized that decline was probably a multi-causal process; in the 1980s these observations found a conceptual framework in the Multiple Stress Hypothesis (MSH), in which predisposing, inciting and (ultimately) contributing factors are thought to combine to cause mortality (Hendershot & Jones 1989). Predisposing factors are held to create a condition in which trees are unhealthy and unable to resist or recover from additional stresses (inciting factors). In the
resultant weakened state contributing factors, singly or in concert, cause death. With the exception of the contentious proposal that acid rain was the major ultimate cause of decline (see Hendershot & Jones 1989), most later authors seem to work, implicitly or explicitly, within the MSH framework.

### 1.7.3 Possible Causes

While a wide array of stress factors have been proposed as responsible for Sugar Maple decline, two see by far the most endorsement: climate (usually drought) and insect defoliation. These factors are mentioned by most authors, both in field studies and in review articles (e.g. Westing 1966; Payette et al. 1996; Houston 1999). A more recent refinement holds that drought or heat stress is most damaging when preceding by a spring freeze (Houston 1999). The proposed mechanism is root death during the freezing event, heightening transpiration stress and causing irreversible vessel cavitation. Auclair et al. 1996) support this hypothesis, arguing that all five major decline episodes during the 20th century in Quebec coincided with severe spring freezing. While attempts to link drought to global warming have proved difficult, the timing and severity of freezing events have been shown to correlate with warming trends (Auclair et al. 1996). None of the papers reviewed here mention either induced drought or heat waves in connection with maple decline. Conversely, insect defoliation is a highly visible and well-established negative impact of tree health, but the status of insect defoliators as inciting or contributing factors remains in doubt. The most commonly identified insect in this regard is the forest tent caterpillar, *Malacosoma disstria* (Hendershot & Jones 1989).
Acid deposition arising from atmospheric industrial pollution, commonly known as 'acid rain', has received much attention as a possible primary cause of decline. Proponents of this hypothesis argue that acid rain (primarily through the deposition of sulfates and nitrates) leaches base cations and mobilizes aluminum ions, negatively impacting tree health (e.g. Duchesne et al. 2005). Soil pH and soil Ca and Al levels have been found to explain roughly a third of the variation in Sugar Maple growth in the second half of the 20th century (Watmough 2002), soil Ca and Mg have been found to be lower on declining as opposed to healthy sites (Liu et al., 1997) and soil acidification has been shown to negatively impact the quality and quantity of symbiotic soil mycobionts (Coughlan et al. 2000). However, disparities in the timing and location of acid deposition and decline have led authors reviewing the literature to conclude that acid rain is not the cause of maple decline, or is at best one factor among many (Houston 1999; Drohan et al. 2002).

In addition to these well studied potential causes of decline a number of other suggestions have been put forwards, both as ultimate (contributing) and proximate (predisposing or inciting) factors. Auclair et al (1996) suggested the 'maturation hypothesis', an elegant argument holding that post-colonization clearing of forests for agriculture and subsequent abandonment created vast, relatively even-aged stands of Sugar Maple in southeastern Canada and the Northeastern United States that were mature / old in the second half of the 20th century (Auclair et al. 1996; Houston 1999). It is argued that older Sugar Maples are more vulnerable to the myriad of stresses associated with decline, and thus the timing and scale of the problem can be viewed as the synchronization of natural processes by past human agency. Since stand maturation may
increase nutrient storage in biomass this hypothesis can also account for altered soil characteristics, such as low Ca values, previously attributed to the leaching effect of acid rain (Watmough 2002). An alternate anthropogenic cause is proposed by Griffin (1965: 299), who argued that "overexposure in residual stands resulting from heavy cutting" was the foremost cause of dieback. Soil compaction (Houston 1999), gradual climatic warming (Griffin 1965) and sudden hot and dry winds (Westing 1966) have also been proposed as causal factors.

1.7.4 Synthesis

Given the number of proposed causes of decline, spatial heterogeneity in occurrence and intensity, and the occurrence of conflicting reports between some sites and times, the MSH is a highly appropriate theoretical tool to combine available information into a coherent theory of decline causes. A simple form of this idea with only two stress stages has been proposed by Houston (1999) but both this theory and the MSH have as yet failed to deliver the sought-after result, that is, a comprehensive suite of management practices capable of reversing decline. This may well be a futile search, as maple decline can arise from a variety of factors that have had varying significance in different locations and at different times. The sudden onset of decline post-1950 could in fact be seen as essentially a stochastic process, with a suite of possible stress factors interacting in a non-predictable fashion at landscape scales to cause decline symptoms. Within this perspective, two major research needs can be identified. Firstly, a meta-analytical approach to decline is required to identify all possible contributing factors, to map their local occurrence, and to explore how they can combine to cause observed
episodes of decline. Secondly, contributing factors must be studied in order to build this meta-analytical picture of decline. Since climate change may cause significant alterations to ecosystem functioning at landscape scales, including but not limited to species invasion, pest and pathogen outbreaks and altered disturbance regimes (IPCC, 2007), substantial work may be required to investigate changing stresses on Sugar Maple that could contribute to decline.

1.8 Preformed and Neoformed Leaves

Leaf preformation and neoformation refer to leaf ontogenetic patterns. Preformed leaves are initiated the year prior to bud break, overwintering within the bud as embryonic leaves and leaf primordia whereas neoformed leaves begin to mature immediately after they are initiated (Steingraeber 1982). Leaf-out in species exhibiting discontinuous growth, such as temperate deciduous trees, may thus be divided into distinct stages: abrupt spring leaf-out as preformed leaves quickly develop, followed (or not) by continuous leaf-out throughout the growing season due to neoformed leaf formation (Lechowicz 1984). For this reason preformed and neoformed leaves are sometimes referred to as 'early' and 'late' leaves, respectively; due to the role of neoformed leaf flushing in response to defoliation (e.g. Heichel & Turner 1983; Ellsworth et al. 1994)) they are also referred to as 'primary' and 'regrowth' foliage. It has been suggested that leaf preformation and neoformation are related to tree successional status and the organization of water-conducting tissues (Lechowicz 1984), but currently very little information on their relevance to plant functioning is available (Guédon et al. 2006).
1.8.1 Preformation and Neoformation in Sugar Maple

Historically, *Acer saccharum* was thought to exhibit preformed leaf growth only (Steingraeber 1982), but beginning in the 1970s numerous authors recognized the occurrence of leaf neoformation in the species. Experimental work on the subject has focused on the role of neoformation as a compensatory response to insect defoliation, observed *in situ* (e.g. Hendershot & Jones 1989), simulated (e.g. Kruger et al. 1998) or induced (e.g. Parker & Houston 1971; Ellsworth et al. 1994). Neoformed leaf production has also been observed to occur naturally in the absence of any apparent stress (Gregory 1980; Steingraeber 1982). Although repeated severe insect defoliation has been observed (Hendershot & Jones 1989), it is not known whether corresponding multiple reflushing events occur; this probably depends on individual tree resource status. In Red Maple (*Acer rubrum*) the date of reflushing and of bud break for next season preformed leaves has been shown to be related to the severity of defoliation, with more severe defoliation resulting in earlier reflushing and increased neoformed leaf production (Heichel & Turner 1983). Naturally occurring neoformed leaf production is probably confined to leader shoots, which have 12-24 leaves on average but sometimes as many as 52, compared to a maximum of 8 for lateral shoots (Steingraeber 1982). These high leaf numbers can be divided into low preformed leaf numbers (4-10; Powell et al. 1982) and larger continuous neoformed leaf production in leader shoots throughout the growing season. Under unstressed conditions, shoots exhibiting both leaf types (termed 'heterophyllous') are more abundant in the crowns of young, vigorous trees, and rarer in slow-growing mature
trees (Gregory 1980); neoformed leaf production has also been observed in saplings (Powell et al. 1982).

Changes in photosynthetic rates of remaining preformed leaves following defoliation, as well as differences in photosynthesis between leaf types, may represent an attempt to compensate for net productivity declines associated with leaf loss. It has been suggested that photosynthetic enhancement (increased photosynthetic rates) may be a common response to leaf loss in perennial species (Heichel & Turner 1983 and see Kruger et al. 1998) although available evidence indicates that photosynthesis in remaining leaf tissue can be either increased or decreased following defoliation (Ellsworth et al. 1994). In Sugar Maple, photosynthesis was been found to be reduced by ~20% in remaining preformed leaves following insect defoliation (Ellsworth et al. 1994), and to be negatively or insignificantly impacted in both neoformed and preformed leaves following simulated defoliation (Kruger et al. 1998). Higher N concentrations in neoformed as opposed to preformed foliage have been observed following simulated defoliation in situ in Red Maple (Heichel & Turner 1983); in this study highly enhanced photosynthetic rates in both preformed (30-60%) and neoformed (~50%) leaves versus controls partially compensated for net assimilation declines.

Morphological differences have been observed between neoformed and preformed leaves in Sugar Maple, with neoformed leaves having significantly larger angles of secondary lobe divergence (calculated as the angle between the mid-vein and the primary vein in the secondary lobe) and more deeply indented sinuses than preformed leaves (Steingraeber 1982). Leaf size and internode length in neoformed leaves have been observed to decline throughout the growing season (Gregory 1980) and following insect
defoliation (Ellsworth et al. 1994; leaf size only). The authors of both studies suggest declining carbohydrate reserves as the causal factor. However, Kruger et al. (1998) found no significant difference in specific leaf area for preformed versus neoformed leaves following simulated defoliation under ambient CO2. While the degree of variability in gross morphological parameters between neoformed and preformed leaves is as yet unstudied, known high levels of variation in leaf shape across the range of Sugar Maple (Desmarais 1952) caution against generalizing these results to the species as a whole.

1.9 Ecophysiology of Acer saccharum

Acer saccharum Marsh., or Sugar Maple, is a common forest species in eastern North America. The tree occurs in Canada in south and central Ontario and Quebec, Nova Scotia, and into the United States (Farrar 1995), having large economic and ecological significance in both countries (Hendershot & Jones 1989; Rebbeck & Loats 1997). It is a common dominant species in mixed hardwood stands throughout its range, and occurs in roughly 45% of the forest area in the Great Lakes - St. Lawrence forest region in Ontario (Thompson 2000). Common associations are with Populus spp (Thompson 2000), Fagus spp. (Cain 1935; Braun 1935; Brewer & Merritt 1978) and as a replacement in stands of Quercus spp. (Kloeppe1 & Abrams 1995). The species has been identified as climax vegetation in Michigan (Brewer & Merritt 1978) and Wisconsin (Struik & Curtis 1962), and as a late successional species in Algonquin Park and the surrounding Great Lakes - St. Lawrence forest region of Ontario (Nichols 1935; Martin 1959), in Quercus-dominated forests in the eastern United States (Kloeppe1 & Abrams 1995), and in north-eastern temperate hardwoods forests in North America more
generally (Braun 1935). Sugar Maple is a large tree, up to 35 m tall, and may live in excess of 200 years (OMNR 2009). Seed production is annual in at least some individuals (Thompson 2000) with especially large crops every 5-7 years (OMNR 2009). Sugar Maple seedlings and saplings have been shown to have intermediate timing of bud break among common eastern upland hardwoods, grown either in the open or under a closed canopy (McGee 1986)

Sugar Maple displays ecophysiological traits well suited to a later seral stage (Kloeppel & Abrams 1995), specifically very high shade tolerance (Thompson 2000; Farrar 1995) and plastic response to limiting light combined with a positive growth response to gap opening (Kloeppel & Abrams 1995). Recent work has shown that this growth response is delayed, absent for two years following gap creation and not reaching a peak until 3-5 years (Jones & Thomas 2004; Jones & Thomas 2007). In a study of seven temperate species Sugar Maple was found to respond most positively to gap creation along with the shade-tolerant Fagus grandifolia (Jones et al. 2009). The authors found a general trend of more shade-tolerant species responding more positively to gap creation, and suggest that differences in relative canopy position and deeper crowns may have allowed shade-tolerant species to exhibit a positive growth response; these findings may therefore not be applicable to the case of individual leaves exposed to increased light intensities.

1.9.1 Heat Tolerance and Adaptation

While no comprehensive analysis of Sugar Maple response to short term acute heat has been undertaken to date, a number of studies provide useful information. In the
only study to expressly consider the impact of high spring temperatures, Hett and Loucks (1971) found that Sugar Maple seedling mortality was not linked to high spring temperatures. However, only more southern populations were examined (S. Wisconsin) in this study and possible genetic variation throughout the species range was not considered. Work on adaptation across an altitudinal gradient in the White Mountains of New Hampshire (Ledig & Korbobo 1983) suggests that Sugar Maple does not show adaptive genetic differentiation in temperature response (no formation of so-called "temperature races"). This conclusion is also supported by the work of Gunderson et al. (2000), who found no evidence of ecotypic adaptation to warming in Northern and Southern provenances. The resistance to spring heat exhibited by saplings in S. Wisconsin may thus constitute a general trait, but it should be noted that temperatures in this experiment were 'above normal' (minimum temperatures greater than 7 °C during April) but did not constitute a 'heat wave' (Hett & Loucks 1971). It is interesting that the White Mountains study found limited genetic capacity for adaptive temperature response, since in general the investigators conclude that the species has the capacity for relatively rapid evolutionary adjustments (Ledig & Korbobo 1983).

While Sugar Maple may not exhibit genetic differentiation indicating adaption to high temperatures, several warming studies have found limited ecophysiologicaclimation responses to moderate temperature increases. Gunderson et al. (2000) examined the effects of a moderate temperature increase on seedlings in the laboratory (daytime temperature of 31 °C versus a control temperature of 27 °C) and field-grown saplings (+4 °C above ambient). The results on productivity are unclear: photosynthetic rates in the treatment groups increased in seedlings by 14% due to thermal compensation,
but declined by an average of 12% in saplings. However, warm-grown seedlings exhibited an upward translational shift in photosynthetic temperature response curves. A similar increase in optimum photosynthetic temperature in heated saplings was non-significant, but may have been obscured by confounding environmental factors. Based on these results, the authors conclude that Sugar Maple has a moderate capacity for physiological acclimation to temperature. Moderate warming (+3-4 °C) of Sugar Maple has also been shown to increase fine root production and mortality (Wan et al. 2004) and to extend the growing season by promoting early bud break and delayed leaf abscission (Norby et al. 2003), but does not change leaf optical properties associated with increased chlorosis (Carter et al. 2000). The implications of these effects on productivity are unclear; for example, productivity gains from an elongated growing season may be offset by the increased risk of damaging early or late frosts (Norby et al. 2003).

While Sugar Maple displays some positive adaptive responses to moderate warming, available evidence does not suggest the tree responds well to acute heat. Huddle and Pallardy (1996) examined the effect of a simulated brush fire (soil and stem base heating of 76.6-85.8 °C for 9.7-10.5 min) on photosynthesis and survival in Acer and Quercus spp. Sugar maple had dramatically lower survival (4.5%) than other species (Acer rubrum - 18.2%, Quercus alba - 48.0%, Q. rubra - 88.0 %). The authors relate this increased mortality to a short-term temporary recovery and high post-fire photosynthetic rates in the two Acer species. Quercus spp. exhibited lower post-fire photosynthetic rates, but had much higher rates of resprouting and survival. These phenomena are interpreted as a delayed and concentrated recovery process in Quercus spp., incorporating abandonment of heat-injured shoots and compartmentalization of damaged tissue in a
longer-term physiological response maximizing survival. Thus, while experimental
temperatures were much higher than ambient air could attain during a weather-related
rather than a fire-related heat event, the physiological responses of Acer and Quercus spp.
suggest that Sugar Maple is poorly adapted to tolerate acute temperature shocks.

Sugar Maple may also be vulnerable to induced drought associated with high
temperature stress. Sperry et al. (1988) found that sugar maple saplings experience
considerable xylem embolisms in winter, preferentially in basal and south-facing trunk
sections, producing an average loss of conductivity of 84% in winter twigs. While these
embolisms were mostly reversible in the spring, this result suggests that the species may
be vulnerable to water stress. This conclusion is corroborated by Martin et al. (1987),
who concluded that Sugar Maple lacks the capacity for significant acclimation to drought
stress based on an examination of electrolyte leakage in excised leaf discs. Both studies
are coherent with the observation that in general shade tolerance is correlated with
decreased ability to withstand heat and water stress (Berry & Bjorkman 1980). Thus,
although conclusive evidence is lacking, extant work suggests that Sugar Maple will
exhibit a strong negative response to the acute heat stress characteristic of heat waves.

1.10 The Focus of this Thesis

This thesis presents the results of an event-driven research effort focused on
improving our understanding of temperate forest responses to acute heat stress. Data were
collected throughout the summer of 2010 at two research sites in Central Ontario
(Haliburton Forest) following three days of very high temperatures in late May (May
25th-27th; see Fig. 2). Critically, Sugar Maple leaves were not fully expanded when heat
shock occurred. Stress symptoms (assessed as leaf discoloration and leaf drop; see Fig. 3 a) and b)) were observed in Sugar Maple and several associated species. Field observations suggested that Sugar Maple sustained the most damage from high temperatures, with Beech (*Fagus grandifolia*) a distant second.

Although high temperatures were preceded by 9 days without precipitation, the total precipitation for May 2010 matches 1971-2000 climate normals (Environment Canada n.d.), suggesting that conditions at Haliburton in mid-May should not be characterized as "drought". However, frosts in early May (nights of May 7th-11th) may have exacerbated the effects of heat later in the month (Auclair et al. 1996; Brodribb & Hill 2000), and the dry spell preceding observed high temperatures could have heightened the physiological effects of heat stress (section 1.2). Visual symptoms of tree damage were observed immediately following the heat event (late May, early June); these included widespread green leaf abscission and leaf browning. Leaf reflushing later in the season (late June) produced a distinct leaf type (neoformed leaves; Fig. 5), in addition to pre-existing and often damaged (preformed; Fig. 4) leaves.

While the effects of high temperatures on forests have been studied, the impact of high temperature stress at a specific phenological stage (during leaf expansion) has not. Expanding leaves may be especially vulnerable to heat stress (Coleman 1986), and thus the May 2010 heatwave represents an opportunity to study how the timing as well as the magnitude of heat impacts may affect forest trees. Furthermore, recent work at the Thomas lab has found strong ontogenetic trends in tree functioning (Thomas 2010), supporting ideas about age/size relationships in tree stress response but challenging some proposed trends (e.g. Boege & Marquis 2005). To contribute to our understanding of
trends in age/size response to stress, leaf loss, damage and reflooding were quantitatively assessed across age classes in Sugar Maple at the site. This constitutes the first *in situ* exploration of ontogenetic variation in heat response in a temperate species across a range of age classes. Assessment of damage and reflooding was also undertaken across canopy gradients to investigate whether observed damage was due primarily to air temperature, light environment, or physiological water deficit. Since temperature acclimation has been observed in both leaf types (section 1.8), fluorescence and morphological measurements were used to investigate plasticity in acclimation to higher temperatures. The availability of a canopy lift (Scanlift240, Finland) allowed a unique opportunity for assessing these traits *in situ* in mature forest trees.

In addition to exploring ontogenetic differences in stress response and recovery, the effect of vertical position in the canopy on high temperature damage and possible leaf-level acclimation to high temperatures, this thesis considers these results within a broader framework of forest response to climate change. Investigating assumptions and uncertainties regarding the effects of spring warming (section 3.1) is critical to advancing our understanding of the impacts of anthropogenic global climate change on both terrestrial carbon cycling and human socioeconomic systems. Consequently, leaf litter collection and gas exchange measurements, both closely related to productivity, were collected throughout the summer of 2010 to indirectly explore the impacts of unseasonal May temperatures on productivity at the study sites.
Chapter 2: Effects of an early season heat wave on ecophysiological parameters related to productivity in Sugar Maple (*Acer saccharum* Marsh.)

2.0 Introduction

Understanding the effects of rising temperatures on forest composition and productivity is of critical importance given current predictions (e.g. IPCC 2007) of the global impacts of climate change. Existing models indicate that an increase in the frequency, duration and intensity of extreme high temperature events (heat waves) is ‘likely’ (Karl & Trenberth 2003; Meehl & Tebaldi 2004; IPCC 2007), and that this increase will be disproportionately large in the northern hemisphere (Meehl & Tebaldi 2004; WGI 2007). Increases in high temperature events are independent of predicted precipitation deficits (droughts), and include short-term acute high temperature events (warm spells, high temperature days and heat waves), which have been observed to increase in frequency across much of Canada since 1950 (Mekis & Vincent 2006). When extreme, heat waves constitute large, infrequent disturbances capable of exceeding unknown ecological thresholds, acting as catalysts for sudden and unpredictable change in forest ecosystems (Turner & Dale 1998). Information regarding heat wave impacts on temperate forests is thus of increasing importance for forest scientists and managers, and for related research interests such as modeling terrestrial carbon sequestration.

Although it is not yet known whether the predicted increase in heat wave occurrence will show seasonal trends, a randomly distributed increase in high temperature events could interact with expected shifts in forest phenology. Advancement
of spring bud-break and greening (Badeck et al., 2004) and a lengthening of the growing season (WGI, 2007) in the mid- and high-latitude regions have been observed throughout the latter half of the 20th century. These changes in forest phenology are predicted to continue with climate change, creating a larger window of opportunity for high temperature events to impact non-dormant trees. Earlier bud-break may also increase the risk of damage from spring frosts (Smith & Cannell 1986; Saxe et al. 2001; Norby et al. 2003), predisposing trees to be vulnerable to heat impacts at the start of the growing season (Auclair et al. 1996). Interactions between the predicted increase in frequency of high temperature climatic events and changing spring phenology could therefore maximize the effects of heat waves during the spring months. The magnitude and nature of these impacts remain largely unknown, as is their potential to counteract positive tree growth responses (Saxe et al. 2001; Goldblum & Rigg 2005) to warming and CO₂ enrichment. Although the impacts of gradual warming on temperate forest productivity and composition have been extensively studied (e.g. Auclair et al. 1996; Huddle & Pallardy 1996; Saxe et al. 2001; Wan et al. 2004) almost no work reports on the effects of short-duration extreme high temperature events on mature trees, stands or forests. Those studies that do consider the effects of transitory high temperatures at larger spatial scales to date have examined only relatively long (i.e. months or more) high temperature events coincident with severe water deficits (Allen et al. 2010). Such events are fundamentally different from short-term heat waves due both to their length and to the dominating impacts of water stress. There is thus a critical gap in our knowledge of how the predicted increase in the frequency of heat waves due to climate change will impact forest form and function.
Although large scale impacts of high temperatures can be predicted from the well-studied molecular processes of acute heat damage and acclimation (for a review see Berry & Bjorkman 1980; Wahid et al. 2007), only broad generalizations have been made so far. Extreme heat damages plant organelles, with the most sensitive responses being changes in chlorophyll protein complexes and the inactivation of heat-labile enzymes (Allakhverdiev et al. 2008), resulting in lowered photosynthetic rates. Threshold temperatures for photosynthetic declines and cellular damage vary between species and are roughly related to the temperature regime to which a species is adapted, as is the potential to acquire thermotolerance (Berry & Bjorkman 1980; Larkindale et al. 2005). Similar broad trends in temperature response are evident at the landscape level, with forests composed of species assemblages less well adapted to high temperatures (e.g. forest types occurring in cool or wet habitats) being generally more vulnerable to the effects of high-temperature-induced drought (Archaux & Wolters 2006). Interspecific differences in heat tolerance (e.g. Berry & Bjorkman 1980; Huddle & Pallardy 1996), the impact of secondary factors such as insect outbreaks (Archaux & Wolters 2006; Roualt et al. 2006), and multi-year time lags in some impact pathways (Ciais et al. 2005; Allen et al. 2010) may also be important determinants of the impacts of high temperature events on temperate forests at larger spatial scales.

A key area that is currently overlooked in the literature pertaining to predicted high temperature responses in trees is the role of ontogenetic variation in high temperature tolerance and recovery. Known ontogenetic trends in resource allocation to defense, growth or reproduction (e.g. Thomas 1996; Weiner 2004; Thomas et al. 2010; Thomas 2010) could drive ontogenetic variation in tree responses to high temperatures by
altering physiological and biochemical characteristics related to damage avoidance (e.g. ratio of leaf to root area, concentrations of photoprotective pigments) or by constraining recovery capacity (reduced resource availability). Previous studies have shown that heat acclimation potential is greatest in early ontogenetic stages (Niinemets 2010) and that younger (e.g. seedlings, saplings) and older (e.g. near-senescent trees) ontogenetic stages are most sensitive to the water stress associated with high temperatures (Archaux & Wolters 2006). However, these results have not been tested across a broad range of age classes. At larger scales, ontogenetic variation in tree-level response to high temperatures could drive changes in cohort structure at the stand level and potentially affect landscape level productivity. Stands exhibiting different cohort structure could display divergent responses to high temperature events. If this variation is large, an accurate understanding of these trends will be critical to any analysis of the impacts of high temperatures on both mixed-aged natural forests and even-aged plantations.

2.0.1 The Role of Event-Driven Research

The lack of research investigating the impacts of extreme heat events at large spatial scales (mature trees and larger) is probably best attributed to practical limitations on experimental manipulation of whole trees or forest ecosystems. Because of these limitations, exploring questions of damage, recovery and acclimation to high temperatures at scales large enough to capture important ontogenetic trends in tree responses requires undertaking event-driven research in the wake of observed climatic phenomena. As causality in environmental phenomena tends to be multi-factor and complex, and the implementation of controls may be impossible, traditional experimental
studies are generally not viable (Hilborn & Ludwig 1993). Consequently it is often necessary to indirectly infer causality following large-scale complex ecological phenomena (Hilborn & Stearns 1982; Plowright et al. 2008). Such inference has proven robust for other event-based research efforts, which in temperate forests have been largely restricted to evaluating the impacts of extreme summer droughts accompanied by high temperatures (e.g. Allen & Breshears 1998; Breshears et al. 2005; Leuzinger et al. 2005; Ciais et al. 2005; Carnicer et al. 2011) and winter storm events (e.g. Duguay et al. 2001; Brommit et al. 2004). In such cases it is critical that both observed major effects (e.g. reduced productivity) and associated effects (e.g. changes in species composition) may be inferentially related to a main cause (e.g. drought), despite uncertainties in the contribution of secondary causes (e.g. slope position). With firm theoretical support such inferences are critical in advancing our understanding of rarely occurring, non-replicable environmental phenomena.

In the specific case of heat impacts, establishing causality is complicated by the physiological mediation of linkages between air temperature and soil water content (e.g., evapotranspiration), which confounds attempts to separate the effects of heat and water stress. Evaporative and evapotranspirative water loss induced by high temperatures can create a water-deficient state in temperate forests (Allen et al. 2010). Subsequent damage such as xylem cavitation (e.g. Sperry et al. 1988; Tyree & Sperry 1989; Breda et al. 2006) is the product of heat-induced water stress rather than the cellular or molecular effects of high temperatures (e.g. destabilization of heat-labile membranes, protein denaturation, etc....). It is therefore necessary to firmly establish heat rather than drought as the primary underlying cause of observed effects before attributing responses to high temperatures.
The role played by the unique precipitation history of any *in situ* observation study site and potentially large uncertainties in quantitatively apportioning observed effects between multiple confounding factors (e.g. heat, drought, soil type, etc...) must also be acknowledged. Qualitative rubrics such as Hill's Criteria (Hill 1965; Plowright et al. 2008) may be helpful in this regard, allowing the identification of a primary underlying cause in a situation with many contributing factors.

### 2.0.2 Focus of This Study

In an effort to address critical gaps in our current knowledge regarding the impacts of acute, short-duration high temperature events on temperate forests, this study reports on key ecosystem parameters in a temperate deciduous tree (*Acer saccharum* Marsh.) following three days of record-setting high temperatures in late May 2010. High temperatures occurred during an intermediate stage of leaf expansion in the study species. Although no formal definition exists, this period was widely described as a heat wave (e.g. CBC News 2010). The term 'short-term acute high temperate event' has also been used in this paper to more accurately characterize the late May heat wave. High temperatures were preceded by a short dry spell and by a mild frost much earlier in the month (see Fig. 2). Immediately following high temperature days widespread abscission of green leaves and leaf scorching were observed in the study species (see Fig 3 a) and b). Reflushing (neoformed leaf production) occurred following leaf loss, but was not continuous throughout the summer. Fieldwork during the summer and fall of 2010 focused on evaluating standard ecophysiological parameters most relevant to damage and recovery processes and productivity. Where possible, comparisons were made against
previously published results from the study site to evaluate differences between 2010 data and 'normal' years. These data were used to address the following key research questions:

1. How did high temperatures impact photosynthetic capacity in Sugar Maple?
2. How did high temperatures affect leaf production in Sugar Maple and associated species in the 2010 growing season?
3. Were trees able to recover from the effects of high temperatures, and what role did the production of neoformed leaves play in their recovery?
4. Did high temperature impacts and recovery vary between ontogenetic stages?
5. Did high temperature impacts and recovery exhibit vertical trends?
6. Were neoformed leaves morphologically, functionally or biochemically distinct from preformed leaves?

2.1 Methods

2.1.0 The Study Site
Data were collected at Haliburton Forest and Wildlife Reserve in central Ontario, Canada (45°13′ N, 78°35′ W) between May and August 2010. Upland hardwood forests in the region are dominated by Acer saccharum Marsh., Fagus grandifolia Ehrh., Tsuga canadensis L. and Betula alleghaniensis Britt. (Thomas, 2010). Average annual temperature for the study region is 4.9 °C with a standard deviation of 0.9, average annual precipitation is 1008.8 mm, the average number of days with a maximum temperature >30 °C is 3.1 and average degree days above 24 °C for May, June and July are 0, 0.1, and 1.8 respectively (Environment Canada n.d.). Fig. 1 presents climate normals for nearby Haliburton town.
The first study site (Kennis Bridge) is located within a conservation zone in Haliburton forest. An existing skid trail network (now used for recreation) allowed sampling with a mobile elevating work platform (Scanlift SL240, Kesla Oyj, 2 Metsolantie, Kesälahti, Finland) across a broad range of age/size classes. Forest in this area is dominated by Sugar Maple. The second study site (Megaplot) is an old-growth forest plot located within Haliburton forest (45°17′ 25″, N 78°38′ 07″ W) and is part of a global network of large-scale forest plots coordinated by the Smithsonian Tropical Research Institution. *Acer saccharum* (upland areas) and *Tsuga canadiensis* (shoreline) are the dominant species in the plot. Litter traps were installed in the megaplot in September 2009. Only litter trap data were collected in the megaplot; all other data were gathered at Kennesis Bridge.

### 2.1.1 The Study Species

*Acer saccharum* is a common dominant in mixed hardwood stands throughout eastern North America, and occupies roughly 45% of the Great Lakes - St. Lawrence forest region of Ontario (Thompson 2000). Sugar Maple is highly shade tolerant (Farrar 1995; Thompson 2000) and exhibits a plastic response to limiting light and a positive growth response to gap openings (Kloeppel & Abrams 1995; Jones et al. 2009). The species has been identified as climax vegetation at several sites throughout its range (Struik & Curtis 1962; Brewer & Merritt 1978). Studies with saplings indicate that Sugar Maple has low heat tolerance (Martin et al. 1987; Sperry et al. 1988; Huddle & Pallardy 1996), and that the potential for acclimation to high temperatures is low (Ledig & Korbobo 1983; Gunderson et al. 2000). While leaves are generally preformed, leaf neoformation has
been observed in the species in response to actual or simulated insect defoliation (Parker & Houston 1971; Hendershot & Jones 1989; Ellsworth et al. 1994; Kruger et al. 1998). No work has yet examined ontogenetic variation in leaf neoformation as a compensatory response, or documented neoformed leaf production in response to high temperature stress.

2.1.2 Litter Traps

60 PVC litter traps were installed in the Haliburton megaplot in September 2009, spaced evenly along a north-south coordinate grid system, with increased density near shorelines. Traps were 0.5 m² and raised roughly 0.8 m above the ground. Traps were collected twice each fall (during leaf fall and when leaf fall was complete) and monthly throughout the summer of 2010 (May, June, July). Leaf samples were dried for 3-4 days (to constant dry mass) at 70 °C and sorted according to species. Sorted samples were weighed using a laboratory scale (Mettler Toledo, PL83-S), accurate to 0.001 g.

Both leaf areas and leaf weights are required for the calculation of leaf area index (LAI). However, leaf area / weight measurements were not made on abscised preformed leaves following the heat event. Consequently, leaf weight (g m⁻²) rather than LAI has been used to report spring leaf loss. While it would be possible to use leaf morphological parameters recorded later in 2010 to calculate spring LAI losses, such an approach would overestimate the magnitude of summer leaf loss, since leaves were not fully expanded when abscised. Because initial leaf loss is most accurately reported as g m⁻², dry mass measurements have also been used to report other collections (fall 2009; fall 2010) to facilitate comparisons. However, LAI is a widely reported ecosystem parameter, whereas
leaf dry mass (g m\(^{-2}\)) are not. To facilitate comparisons of observed changes with other work, changes in end of year LAI for Sugar Maple were calculated for 2009 and 2010. LAI was calculated as LAI = \((1/\text{leaf mass per area})*\text{dry weight of collected leaves})/\text{trap area}, on a per-trap basis. LAI calculations for Sugar Maple in 2010 used a weighted average of leaf mass per area (neoformed leaves= 33.6%, preformed leaves= 66.4% of total leaf production, averaged across all canopy levels and size classes) to account for differences in leaf morphology between leaf types.

2.1.3 Twig Counts

Work in June focused on evaluating preformed leaf loss and neoformed leaf production across size/age classes. Four categories of trees were sampled: saplings (dbh < 2cm), small trees of reproductive age (Thomas 2010) with exposed crowns (dbh 17-30 cm), medium sized canopy dominant or co-dominant trees (dbh 31-54 cm), and very old, possibly senescent trees, also with dominant canopy positions (dbh > 55 cm). Crown exposure was evaluating following Clark and Clark (1992). All trees except saplings had a crown exposure rating of either 4 or 5 according to the crown illumination index presented by those authors (≥90% of the vertical projection of the crown exposed to vertical light; some (4) or all (5) of the 90° inverted cone encompassing the crown exposed to lateral light). All saplings occurred in gaps and had unobstructed vertical access to light. Only healthy trees were selected for sampling: severe crown damage, obvious disease, or heart rot were grounds for exclusion. These criteria, combined with limited availability of lift-accessible trees, prevented a fully symmetrical sampling design, with 15, 11, 14 and 15 individuals sampled for saplings, small, medium and large
trees, respectively. Locating small mature trees with adequate crown exposure was the major constraint on sample size.

Selected trees were sampled using the mobile elevating work platform (MEWP) at three visually assessed canopy levels (upper, mid, and lower-canopy). Tree crowns were divided into three roughly equal vertical layers, but sampling at upper and lower levels was undertaken as close to the upper and lower perimeters of the crown as possible in order to maximize the effect of vertical position on collected data. Stray branches occurring below the bulk of the crown were not considered to constitute part of the lower canopy. Mid canopy sampling was undertaken as close as possible to the midpoint of the crown (equidistant from upper and lower perimeter). Since shoot position (leader versus lateral) has been shown to affect neoformed leaf production in Sugar Maple (Steingraeber, 1982), only leader shoots were sampled. In those cases where leader shoots were not distinguishable despite extensive surveying of the canopy stratum (notably at the lower level in some medium and large trees) a lateral shoot was randomly selected.

At each canopy level the most recent year’s growth (one internode) was sampled from each of three shoots, selected using a random numbers table. The MEWP basket was positioned within reach of a tree crown level, and a random number was used to count along accessible shoots, starting with the shoot closest to the MEWP basket. The selected shoot (i.e. the $n$th shoot from the basket) was cut and transported to a field station for leaf counting. Leaves in Sugar Maple are opposite and display prominent bud scars. Missing preformed leaves could therefore be counted as paired bud scars or missing leaves opposite retained preformed leaves. Preformed leaves were assigned to
one of four visually assessed leaf scorch classes (0%, <20%, 20-50%, and >50% of leaf area scorched). The following indices were calculated from raw data: the ratio of neoformed leaves produced to preformed leaves produced (N_R), the ratio of neoformed leaves produced to preformed leaves lost (N_L), and scorch score (S_S). Scorch score was calculated as \( S_S = ((\text{number of leaves showing } <20\%\text{ scorch} \times 10) + (\text{number of } 20-50\%\text{ scorch} \times 35) + (\text{number of } >50\%\text{ scorch} \times 75))/(\text{total number of scorched leaves} \times 100)) \). This last metric provides a weighted average of leaf scorching.

### 2.1.4 Gas Exchange

Gas exchange measurements were restricted to light-saturated photosynthesis (\( A_{\text{max}} \), calculated on a per area basis) as unstable weather conditions and low photosynthetic rates made the collection of data on photosynthetic response to CO\(_2\) (\( A/C_i \) curves) or light (light response curves) too slow to collect with an adequate sample size. \( A_{\text{max}} \) can be efficiently surveyed, is commonly reported, and is closely related to integrated carbon uptake in forest canopies (Thomas 2010) as well as constituting "an index of integrated natural constraints on photosynthesis" (Field & Mooney 1983:49). It is thus an ideal metric both for exploring the effects of heat on photosynthetic capacity in Pre and Neo leaves and for examining the implications of these effects in relation to other studies.

\( A_{\text{max}} \) measurements were taken at upper and lower canopy levels on sun-exposed leaves between 0800 and 1300h to avoid the strong down-regulation of photosynthesis observed in afternoon measurements. Measurements were undertaken using a LI-6400 XT photosynthesis system (Li-Cor, Lincoln, NE) equipped with a red/blue light source (6000-02B Red-Blue SI-0951). Leaves were maintained at a CO\(_2\) concentration of 350
ppm, relative humidity of 50-80%, leaf temperature of 20-25 °C (vapor pressure difference (VPD) of 0.5-1.6 kPa) and a photosynthetic photon flux density (PPFD) of 1000 µmol m⁻² s⁻¹. This level of PPFD has been shown to be saturating for Sugar Maple (Thomas 2010), a result that was tested and confirmed with several light response curves at the start of sampling.

While $A_{\text{max}}$ survey measurements were taken in late July and August, earlier measurements were desired to examine seasonal changes in photosynthetic capacity following heat. Consequently $A_{\text{max}}$ at 350 ppm CO₂ was extracted by fitting linear functions (due to anticipated high errors in the data) to $A/C_i$ curves recorded in early July. The model was selected to force the linear function through the origin. Data from 2010 was compared against $A_{\text{max}}$ measurements taken in previous years at the same site, in some cases on the same trees (see Jones and Thomas, 2007; Thomas, 2010; Patankar et al., in press). Conditions under which data were collected varied slightly; tolerance ranges were therefore established of +/- 2 µmol m⁻² s⁻¹ for PPFD and -10/+30 ppm for CO₂. Jones and Thomas (2007) and Patankar et al (in press) both found treatment effects for sampled trees and consequently only control trees from these studies were included. Since a strong unimodal (hump-shaped) relationship in $A_{\text{max}}$ versus dbh has been observed in the study species, with $A_{\text{max}}$ calculated on a per area basis observed to peak near the size of reproductive onset (14.2 cm dbh; Thomas, 2010), only data from trees with dbh 25-55cm were included.

Since $A_{\text{max}}$ and associated parameters (stomatal conductance $g_s$, water use efficiency WUE), leaf carbon content and leaf morphometrics (see below) are expected to covary, an ANCOVA using leaf type and canopy stratum as predictor variables was used
to assess overall significance in the dataset. Year was not included as a predictor variable because all leaves from previous years were of the same type ('standard' year leaves). Testing for a response of any given variable as a function of leaf type, canopy stratum and year confounds the effects of leaf type and year, artificially lowering the significance of both factors. Note, however, that testing for differences between standard year, neoformed and preformed leaves is equivalent to pooling prior years data, and testing for differences between the two leaf types observed in 2010 versus a composite standard year. Since the composite year captures the variability between years, stochasticity in measured leaf characteristics in the absence of an early-season heat event is included and it is possible to test whether neoformed or preformed leaves are truly different from standard year leaves observed in normal years.

2.1.5 Fluorescence

Fluorescence data were collected in late July and early August using a mini-PAM Photosynthesis Yield Analyzer (Heinz Walz GmbH, Effeltrich, Germany). As the name suggests, the mini-PAM utilizes pulse amplitude modulation to accurately assess fluorescence signals against background signals of up to $10^6$ greater magnitude (Heinz-Walz, 1999). This is done by switching measuring light on and off at high frequency and timing the detector to register only fluorescence excited by the measuring light (for full details see Schreiber, 2004). The mini-PAM also applies a short-pass filter (<670 nm) to the (actinic) measuring light source, and a long-pass (>700nm) on the detector.

Trees selected for fluorescence measurements were an arbitrarily selected subset of trees sampled for gas exchange and twig counts (dbh) but no attempt was made to
match fluorescence and other measurements on the same leaf. The most informative fluorescence analyses require careful repeated measurements on the same leaf in both light and dark-adapted states, and as Maxwell and Johnson (2000:664) acutely observe, "...if experiments are not designed correctly, [fluorescence] can be impossible to interpret". Furthermore the need to accurately assess dark-adapted fluorescence "...can be a major limitation in field conditions" (Maxwell & Johnson 2000: 661). Both these considerations limited analysis of collected fluorescence data, with inappropriate design and inadequate dark-adaptation techniques rendering much of the data useless. Accurate measurements were obtained of truly dark-adapted pre-dawn measurements made on three trees (dbh 20-30 cm) after at least 12 hours of darkness. Measurements were made at upper and lower canopy positions, and leaves were sampled at two canopy positions with repeated measurements made on non-overlapping areas of the same leaf for each of three leaves of both types (neoformed and preformed). Dark-adapted measurements were used to calculate the maximum quantum yield of photosystem II, \( F_v/F_m = (F_m-F_o)/F_m \) where \( F_m \) is maximum and \( F_o \) is minimum chlorophyll fluorescence in the dark-adapted state (Maxwell and Johnson, 2000).

2.1.6 Leaf Optics

Leaves used for optics measurements were collected between August 1st and 9th in order to minimize potential bias arising from leaf ontogenetic changes throughout the summer season. Leaf spectral reflectance and transmittance were simultaneously measured using a custom-built dual integrating sphere system (components from Ocean Optics, Inc., Dunedin, Florida, USA: for a detailed description see Baltzer and Thomas, 2005). The
system consists of two spheres, one containing a light source, in between which leaves are clamped. Since the interior surfaces within each sphere exhibit Lambertian reflectance, light collected with fiber optic cables attached separately to each sphere can be assumed to be a randomly mixed and representative sample of light either reflected from the leaf surface (upstream sphere containing measuring light source) or transmitted through it (downstream sphere). Fiber optics connect to a spectroradiometer accurate to ~0.3 nm. To correct for imperfections in the system (e.g. ambient light entering the spheres) reflectance was calculated as \((R_s-R_d)/(R_r-R_d)\), where \(R_s\) is the light reflected from the leaf surface, \(R_d\) is the dark reference (taken daily with the measuring light source switched off and no leaf placed between the spheres) measuring ambient light penetrating the instrument, and \(R_r\) is the output from the upstream sphere when a white reference standard (model NT54-302, Edmund Industrial Optics) is applied. Similarly spectral transmittance was calculated as \((T_s-T_d)/(T_r-T_d)\), where \(T_s\) is transmittance of light through the leaf sample, \(T_d\) is the dark reference, and \(T_r\) is the output of the downstream sphere with the measuring light source switched on but no leaf placed between the spheres. Absorbance was calculated as \(A=1-(R+T)\).

Empirical optical indices were calculated from cleaned data to quantify relative amounts of major pigment groups (chlorophylls, anthocyanins, carotenoids) between leaf types and canopy strata. Chlorophyll was examined using the modified Simple Ratio (mSR = \((R_{728}-R_{434})/(R_{720}-R_{434})\), where \(R_x\) is reflectance at wavelength \(x\)), which has been found to be best correlated to measured chlorophyll content in Sugar Maples at Haliburton (Zhang et al. 2007). Because of instrument noise in the near infrared, the Anthocyanin Reflectance Index (Gitelson et al. 2001; \(ARI = R_{550}^{-1}-R_{700}^{-1}\)) was used to
estimate anthocyanin content; this index has been found to accurately estimate anthocyanin content across multiple species with no re-parameterization of algorithms (Gitelson et al. 2009). Carotenoid content was assessed using a Carotenoid Reflectance Index \( (\text{CRI}_{550} = R_{510}^{-1} - R_{550}^{-1}) \), which includes reflectance at 550 nm (to remove the effect of chlorophyll) but does not utilize measurements from the near infrared (Gitelson et al. 2002).

### 2.1.7 Leaf Morphometrics

All leaves on which gas exchange and fluorescence measurements were made were weighed and measured to quantify standard morphological parameters (leaf mass, leaf area, leaf mass per area) for preformed and neoformed leaves across canopy strata. Immediately after harvesting leaves were scanned and leaf area was determined using the public domain program ImageJ (version 1.44 for Mac, National Institute of Public Health, Maryland). Leaf laminar thickness was measured using a low-force micrometer (No. 227-101, Mitutoyo Co., Japan), and three punches were then taken from each leaf using a standard single-hole punch, avoiding major veins. Leaves and punches were dried for 2-4 days in a domestic food dehydrator (American Harvest, Snackmaster Dehydrator) at 60 °C and weighed in a semi-microbalance (accurate to +0.00001). Punch weight was averaged on a leaf-by-leaf basis. Leaf mass per area was calculated as total leaf dry mass/leaf area. Leaf mass per area of the lamina tissue only (LAI\text{lamina}) was calculated as dry mass of leaf punches / leaf punch area.
2.1.8 Carbon / Nitrogen Analysis

Paired sets of dried neoformed and preformed leaves sampled during gas exchange and fluorescence measurements were analyzed using an ECS 4010 analyzer (Costech Analytical Technologies, Valencia, CA) to determine percent C and N content. Trees from which leaves were selected had an average dbh of 31 cm, with a minimum of 19.3 and a maximum of 56.1.

2.1.9 Data analysis

Analysis was conducted using the open-source statistical program R (The R Foundation for Statistical Computing, 2010: version 2.11.1). Where multiple samples were collected from individual trees (e.g. leaf count data, fluorescence measurements, gas exchange measurements), data were pooled by leaf type and canopy stratum to avoid pseudo-replication. Prior to analysis all data were assessed for normality using a normal Q-Q plot. Due to observed leptokurtosis in leaf count data the Anscombe-Glynn test for kurtosis (Anscombe & Glynn 1983) was applied to all leaf-count-derived metrics prior to analysis. Leptokurtic data were square-root transformed to fulfill the criteria of normalcy prior to statistical testing.

Individually collected datasets were compiled so that related variables were not analyzed in isolation (e.g. gas exchange and morphological data were compiled). For each compiled data set, analysis proceeded in a hierarchical order, beginning with a MANOVA or ANCOVA (table 2) including all predictor and response variables. Terms not found to be significant when all variables were included were then excluded, and multiple two-way ANOVAs were used to explore significance in individual response
variables. Tukey's Honestly Significant Difference was used to explore significant differences in the distribution of each response variable across all levels of the predictor variables.

High levels of noise in optics data (most likely due to a non-stable power supply to the field laboratory) required more extensive post-processing than other data sets. Spectra containing more than half theoretically impossible values (A, R or T <0 or >1) were removed from the analysis. Data were averaged by leaf type, canopy level, and metric, a recursive moving average (frame size=10) was applied to smooth data at 550 nm and below, and a Lowess curve (f=0.01) was plotted for visual clarity (Fig. 15). Significant differences between leaf types at each canopy level were evaluated using a T-Test (two tailed, homoscedastic). Since data points were very dense only large regions of significant difference (α=0.05) have been reported in Fig. 15. In addition, because a large number of samples had to be dropped from the analysis due to instrument noise it was not possible to pool data by tree, leaf type and canopy strata prior to testing for significance.

2.2. Results

2.2.1 Litterfall and LAI

Tabulated dry leaf mass for all species for which summer leaf abscission was observed are presented in Fig. 6. May litter collection was much larger in Sugar Maple (7.07 g m\(^{-2}\)) than in all other species (\textit{Acer rubrum} - 0.51 g m\(^{-2}\), \textit{Betula cordifolia} - 0.07 g m\(^{-2}\), \textit{Fagus grandifolia} - 0.15 g m\(^{-2}\), \textit{Prunus serotina} - 0.003 g m\(^{-2}\)), but Sugar Maple greatly outnumbers these species at the megaplot. May litter collection was therefore calculated as a percentage of total 2009 litter production (since 2010 leaf count data for all species...
was not fully tabulated at the time of writing), revealing that summer litter fall in Sugar Maple (6.69%) was proportionally much higher than in all other species (average-0.93%). Total 2010 litter production (g m$^{-2}$) for Sugar Maple was reduced by 33% compared against 2009, the only previous year for which data are available. The decline in Leaf Area Index (LAI) was much larger. Converting litter production to LAI (Fig. 8) shows LAI declines in the Megaplot (Sugar Maple only) of 60.02% between 2009 and 2010 (end of year cumulative totals).

### 2.2.2 Leaf Mortality, Leaf Scorching and Neoformed Leaf Production

Previous studies on Sugar Maple have reported preformed leaf numbers of 4, 6 or 8 (Steingraeber 1982); our results confirm these numbers with the exception of samples taken from the tops of large size class trees, which had an average of 9.04 ±SE 1.46 leaves with a maximum of 30. High preformed leaf numbers were also observed in the tops of small trees, which had a maximum of 14. Leaf count metrics derived from preformed and neoformed leaf numbers and scorch measurements are displayed in Fig. 9.

On average, 67% of preformed leaves that survived the heat event displayed some level of scorching. Average severity of leaf scorch was moderate ($S_{5}=0.33$), with 20-50% of the total leaf area of most leaves scorched. Scorch score and percent of leaves scorched did not vary significantly across canopy positions or age classes. Leaf loss increased with height, with the upper canopy strata having significantly more leaves lost per internode (average=2.53) than either the middle (1.65) or lower (1.06) canopy. Vertical trends in leaf loss were found to be significant when expressed as percent mortality of preformed leaves (upper canopy=40.4%, middle-canopy=29.2%, lower canopy = 20.5 %). Among
age classes leaf mortality expressed both as absolute numbers of lost leaves and as percent mortality was significantly lower in saplings than in mature trees.

Neoformed leaf production increased strongly with increasing canopy height, although this increase was not as large in the upper canopy of large trees as in small or medium individuals. Saplings exhibited much lower leaf production than all other trees at all canopy levels. The numbers of neoformed leaves produced per preformed leaf lost and the ratio of neoformed:preformed leaves (\(N_R\)) were significantly higher in the upper canopy than in all other canopy levels. \(N_R\) was also significantly higher in the middle canopy than the lower. Tukey's HSD revealed that both metrics did not exhibit significant differences between size classes of mature trees, but were significantly lower in saplings (\(N_R = 0.14\), \(N_L = 0.43\)) than in mature trees (average \(N_R = 1.62\), average \(N_L = 3.77\)). A significant interaction term (class×level) for \(N_R\) and \(N_L\) indicates a significant effect of tree size on canopy-level leaf production (Fig. 9 b,c). Significance was driven by the deviation of saplings from all other size classes at all canopy levels, but low neoformed leaf production in the upper canopies of large trees (seen in both \(N_L\) and \(N_R\) scores) is also noteworthy.

**2.2.3 Gas-exchange**

Gas-exchange data is summarized in Figs. 8 and 11; results from analysis of variance are presented in Table 7. Note that early season preformed and neoformed leaves are denoted as Pre and Neo leaves, respectively. While preformed leaves exhibited significantly reduced photosynthetic capacity versus standard year leaves (\(\text{Photo}_{\text{avg}} = 5.3\) \(\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}\) vs 6.8 \(\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}\), Tukey's HSD \(p_{\text{adj}} = 0.0037\)) no significant
differences were observed between neoformed (\(\text{Photo}_{\text{avg}}=6.0 \ \mu\text{mol CO}_2 \ \text{m}^{-2}\ \text{s}^{-1}\)) and standard year or neoformed and preformed leaf types. Average photosynthetic capacity for early season 2010 measurements (\(\text{Pre}_c=2.4 \ \mu\text{mol CO}_2 \ \text{m}^{-2}\ \text{s}^{-1}\), \(\text{Neo}_c=2.4 \ \mu\text{mol CO}_2 \ \text{m}^{-2}\ \text{s}^{-1}\)) was significantly lower than for preformed, neoformed and standard year leaf types (\(\text{Pre}_c\) vs preformed, \(p_{\text{adj}}=0.0179\); \(\text{Pre}_c\) vs neoformed, \(p_{\text{adj}}=0.0013\); \(\text{Pre}_c\) vs standard year, \(p_{\text{adj}}<0.001\); \(\text{Neo}_c\) vs preformed, \(p_{\text{adj}}=0.0074\); \(\text{Neo}_c\) vs neoformed, \(p_{\text{adj}}=0.0003\); \(\text{Neo}_c\) vs standard year, \(p_{\text{adj}}<0.001\)). No significant effect of canopy stratum or of leaf type\(X\)level was observed. Conductance rates for neoformed leaves (\(\text{cond}_{\text{avg}}=0.1276 \ \mu\text{mol H}_2\text{O m}^{-2}\ \text{s}^{-1}\)) were significantly higher than observed conductance in preformed (0.0810, \(p_{\text{adj}}<0.001\)) or standard year (0.0829, \(p_{\text{adj}}<0.001\)) leaves. No significant differences were observed between standard year and preformed leaves or between canopy strata. The interaction of type\(X\)level was not significant. Note that conductance data were not averaged or tested for \(\text{Pre}_c\) and \(\text{Neo}_c\) leaves as these measurements are reconstructed. No significant differences in water use efficiency (WUE) were observed between leaf types or canopy strata.

### 2.2.4 Fluorescence

A two way ANOVA using leaf type and canopy level as predictor variables found a significant effect of leaf type on the maximum quantum yield of photosystem II, \(F_v/F_m\), in the dark-adapted state (\(p=0.0048\), \(\text{Pre}_{\text{avg}}=0.8224\), \(\text{Neo}_{\text{avg}}=0.8056\)). These results are presented in Fig. 12. Although mean \(F_o\) was higher in neoformed than in preformed leaves (\(\text{Neo}_{\text{avg}}=311.6549\) vs \(\text{Pre}_{\text{avg}}=292.3222\)) this difference was not significant. No significant differences across canopy levels were observed for \(F_o\) measurements.
2.2.5 Leaf Optics

Differences between neoformed and preformed leaves were non-significant for all calculated indices. Compiled absorbance, reflectance and transmittance spectra are presented in Fig. 15. Values for calculated optical indices are reported in table 2.

2.2.6 Leaf C/N content

Leaf carbon content was significantly higher in standard year leaves (48.6%) than in neoformed (45.1%) or preformed leaves (45.3%, \( p_{\text{adj}}<0.0001 \) in both cases) but no differences were found between neoformed and preformed leaves (see Fig. 14). Upper stratum leaves had significantly higher carbon content than low leaves (46.7% vs. 44.9%, \( p_{\text{adj}}=0.0231 \)) but since the interaction term typeXstratum was not significant, differences between leaf types at different levels cannot be tested. Significant differences in leaf nitrogen content were observed between neoformed and preformed leaves (\( p_{\text{adj}}<0.001 \)) and between neoformed and standard year (\( p_{\text{adj}}<0.001 \)) but not between standard year and preformed leaves. Leaf nitrogen content was lower in neoformed leaves (avg=1.7%) than either preformed (2.1%) or standard year leaves (2.2%). No significant effect of canopy strata was found.

2.2.7 Leaf Morphometrics

Results of leaf morphometric measurements are presented in Fig. 13. Leaf mass was highest in standard year leaves (mean=0.4805g), followed by preformed (0.3251g) and neoformed (0.2068g) leaves; differences in leaf mass were found to be significant in all
cases (preformed vs neoformed, \( p_{adj}=0.0358 \); standard year vs neoformed, \( p_{adj}<0.0001 \); standard year vs preformed, \( p_{adj}<0.001 \)). The effect of canopy position on leaf mass was not significant. Differences in leaf area were significant between standard year (mean=67.85 cm\(^2\)) and neoformed (mean=29.75 cm\(^2\)) leaves (\( p_{adj}<0.0001 \)) and between standard year and preformed (mean=40.20 cm\(^2\)) leaves (\( p_{adj}<0.0001 \)), but not between neoformed and preformed leaves. Again, canopy strata had no significant effect. LMA was significantly different between leaf types (\( p<0.001 \)) and canopy strata (\( p<0.001 \)). Tukey's HSD showed that while neoformed leaves had a significantly lower LMA than both preformed (69.20 vs 85.33, \( p_{adj}=0.0001 \)) and standard year (vs 83.8247, \( p_{adj}=0.0008 \)) leaves, no significant difference was observed between standard year and preformed leaves. Upper stratum leaves had a significantly higher LMA than low (87.44 vs 63.92, \( p_{adj}<0.0001 \)), but the lack of a significant interaction term (type x stratum) prevents testing of differences in LMA across both leaf types and canopy strata. LMA\(_{lam}\) was significantly higher for preformed leaves (avg=92.1) than neoformed (avg=75.4, \( p_{adj}<0.001 \)) and standard year (avg=66.9, \( p_{adj}<0.001 \)) leaves, but no significant difference was observed between standard year and neoformed leaves. Canopy position had a significant effect on LMA\(_{lam}\) (high avg=87.5, low avg=66.1, \( p_{adj}<0.0001 \)). The interaction term was not significant.

2.3 Discussion

High temperatures in late May 2010 substantially impacted photosynthetic capacity in Sugar Maple. Light saturated photosynthetic rate (\( A_{max} \)) was significantly reduced for much of the 2010 growing season, in both damaged preformed leaves and developing
neoformed leaves. Large and unprecedented leaf drop also reduced the quantity of photosynthetic material (leaf production, total leaf mass and LAI) relative to a normal year (2009). Trees appeared to almost fully recover physiologically ($A_{\text{max}}$, WUE) from the effects of high temperatures, producing large flushes of neoformed leaves to compensate for preformed leaf abscission. However, delays in recovery times and the inability to fully replace lost leaf material suggest that productivity in Sugar Maple was severely reduced over the entire 2010 growing season as a consequence of high temperature stress. The degree to which increased productivity in other less sensitive species may have compensated for this reduction at the ecosystem level remains to be explored.

High temperature impacts (leaf loss, scorching) and recovery (reflushing) varied significantly between ontogenetic stages. Saplings were less severely impacted than canopy trees (less leaf loss). High temperature effects were likely mitigated in saplings due to a lower degree of exposure in the forest understory. However, saplings also showed a dramatically lower ability to reflush. Reduction and recovery of $A_{\text{max}}$ was not assessed across ontogenetic stages. Leaf reflushing was concentrated in the upper canopy in all size classes, with the exception of largest size class trees. In these individuals, reduced vascular connectivity in the upper canopy may have constrained reflushing in this most optimal canopy position. Neoformed leaves were morphologically distinct from preformed leaves, but functional differences between leaf types had largely disappeared by late summer. Neoformed leaves had significantly lower nitrogen concentrations than preformed leaves. Morphological measurements suggest that neoformed leaves may have
been acclimated to higher temperatures (see 2.3.7), but enhanced photoprotection was not observed.

2.3.1 Leaf Loss

The May heat wave caused large-scale summer leaf abscission in Sugar Maple (Fig. 6; Fig. 3a). Mature trees lost an average of 33.8% of preformed leaves, compared to 19.2% for saplings. Leaf loss was greatest in the upper canopy for all size classes (see Fig. 9). Decreased leaf loss in saplings and in the lower canopies of mature trees is consistent with known abiotic gradients in light intensity and temperature in forest canopies and understories (Naidu & DeLucia 1997; Niinemets & Valladares 2004; Niinemets 2007; Nock et al. 2008), suggesting that both leaf heating and photodamage may have caused leaf abscission. Scorching of surviving leaves was fairly low (21.7% in mature trees) and leaves tended to be moderately scorched ($S_{S\,\text{avg.}} = 0.35$ in mature trees). Scorching displayed no vertical trend. However, because abscised leaves were often scorched, higher rates of leaf abscission in the upper canopy would have obscured any trend in scorching. Field observations suggest that sun exposure determined leaf scorching in the lower canopy and understory.

Abscised leaves were still green and appeared to have fallen on high temperature days, suggesting that leaves were actively shed in an attempt to control evapotranspirative deficits. Leaf shedding in response to drought has been suggested as a cost-effective means for plants to avoid drought stress and xylem cavitation by segmentation (Tyree et al. 1993). Furthermore, shedding may maintain higher leaf water potential and conductance in remaining leaves (Ginter-Whitehouse et al. 1983). It has
been suggested that small stems (twigs and fine branches) in Sugar Maple are preferentially vulnerable to cavitation in order to conserve vascular connectivity in larger stems (larger branches, trunk), which represent more substantial resource investments (Tyree et al. 1991). However, the results of this study (immediate and large scale neoformed leaf production on the most recent years growth) are not consistent with widespread xylem cavitation in fine stems.

2.3.2 Reductions in Photosynthetic Capacity

Preformed leaves surviving the late May heat wave had severely reduced photosynthetic capacity relative to leaves from 'normal' years. Average $A_{\text{max}}$ in previous years was 6.89 $\mu$mol CO$_2$ m$^{-2}$s$^{-1}$; $A_{\text{max}}$ in preformed leaves measured in June (P$_e$ leaves) averaged 2.44 $\mu$mol CO$_2$ m$^{-2}$s$^{-1}$, a decrease of 65%. Studies have suggested that leaf ontogenetic trends in photosynthetic capacity peak at or near full leaf expansion (Freeland 1952; Ludlow & Wilson 1971; Sestak et al. 1985; Coleman 1986), but later peaks have also been observed (e.g. Choinski Jr et al. 2003). If post-peak decline is gradual photosynthetic capacity may remain constant throughout the summer months, but this trend has not been shown for trees at the Kennisis Bridge study site. Therefore, it is possible that some of the observed reduction in photosynthetic capacity in 2010 is attributable to differences in sampling times, as most pre-2010 measurements were made (on average) later in the season (July / August). However, measurements from previous years that relied heavily on early season (June) measurements (e.g., Patankar et al., 2011) were still significantly higher than $P_e$ $A_{\text{max}}$ measurements, suggesting differences in average sampling time (early season 2010 measurements vs. prior years) do not explain the reduction in $A_{\text{max}}$ observed in $P_e$ leaves.
Sustained decreases in $A_{\text{max}}$ have been linked to heat and drought events (e.g. Leuzinger et al. 2005) and are predicted from established mechanisms of heat damage to the photosynthetic apparatus (for a review see Berry & Bjorkman 1980; Breda et al. 2006; Wahid et al. 2007). Decreased photosynthetic capacity in early season preformed leaves ($P_e$ leaves) is thus best explained as the result of high temperature-induced damage accumulated during the May heat wave.

2.3.3 Contributing Factors

Establishing causality in any event-based research effort focused on climatic phenomena is complicated by the tendency of such phenomena to be unique. Variations in the magnitude and characteristics of contributing factors, their temporal sequence, and pre-existing conditions at the study site constrain attempts to accurately apportion observed effects amongst potential contributing factors. However, strong qualitative arguments can still be made, and indeed must be made in order to advance our understanding of these non-replicable phenomena.

Frost in early May could have predisposed sampled trees to heat damage (Auclair et al. 1996), but historical climate data suggests that the frost event is not a satisfactory explanation for observed leaf drop. During the period 1971-2000 May saw an average of 6 days on which temperatures dropped below 0 °C at Haliburton, and 1.5 on which they dropped below -2 °C (Environment Canada 2011). May frosts are thus normal events at the study site. These frosts have not led to leaf abscission in prior years. Furthermore, frost occurred prior to leaf expansion and abscised leaves did not display the blackened appearance characteristic of frost damage. Similarly, while the effects of a short dry spell
(May 15th-May 26th) cannot be separated from high temperature impacts, precipitation deficits do not explain leaf fall or suggest that heat should not be considered as its main driver. May precipitation has been recorded at similar levels in previous years (May average in 2010 = 92.2mm, May average from 1971-2000 = 92.8 mm), but previous years did not experience either record-setting temperatures or leaf drop (P. Schleifenbaum, pers. comm.). While early May frost and mid-May precipitation deficits may have predisposed trees to high temperature stress or exacerbated damage, high temperatures in late May stand out as the only potential causal factor specifically associated with leaf abscission.

The hypothesis that high May temperatures caused leaf drop is strongly supported by a qualitative analysis using a modified version of Hill's diagnostic criteria (Hill 1965; Plowright et al. 2008). Originally developed for epidemiological applications, Hill's criteria allow the rigorous evaluation of the relationship between an observed effect and a suggested cause even experimental testing is impossible (although experimental evidence can be incorporated within this diagnostic framework). A comprehensive list of criteria is presented in Table 1. Use of Hill's criteria is intuitive: the more criteria matched by a suggested causal factor, the stronger the association is between that factor and observed effects. Taking high temperatures as the cause of leaf drop fulfills the criteria of specificity (spring/summer leaf drop was not seen in the absence of heat), temporality (heat preceded leaf mortality), biological response gradients (leaf mortality varied positively with expected temperature gradients throughout the canopy), coherence (explanation is in accordance with current body of knowledge), consistency (heat was associated with leaf drop temporally and spatially - unpublished data) and plausibility
(heat is an ecologically sensible explanation for observed leaf drop). Both frost and drought fail to fulfill the criteria of specificity, biological response gradients and consistency, suggesting that neither cause is as good an explanation for observed effects as high temperatures in late May.

2.3.4 Reflushing and Recovery

Leaf neoformation in Sugar Maple occurs when growth of epicormic buds is released, possibly from hormone inhibitors produced in existing preformed leaves (Gregory 1980b). Reflushing has been observed in Sugar Maple following actual and simulated insect defoliations (Parker & Houston 1971; Hendershot & Jones 1989; Ellsworth et al. 1994; Kruger et al. 1998), but refushing responses have only been quantified for seedlings and saplings. The dynamics of recovery-response refushing at stand and ecosystem scales are thus largely unexplored. In this study we observed a large refushing response in mature trees, and a restricted response in saplings (Fig. 9). Saplings displayed lower leaf mortality than mature trees and as a result comparisons of absolute neoformed leaf production are misleading. However, refushing vigor can be assessed as the ratio of neoformed leaves produced per preformed leaf lost. Mature trees on average produced 4.31 neoformed leaves for every preformed leaf lost compared to 0.5249 for saplings, suggesting that relying on sapling experiments to examine the response of Sugar Maple to defoliation may severely underestimate the magnitude of the compensatory response.

Despite large-scale refushing total (end of season) litter production for Sugar Maple in 2010 was 70.76 g/m² versus 105.55 g/m² in 2009, a reduction of 32.96%. When converted to LAI this reduction was almost doubled, producing a decrease in LAI of
~60% between 2009 and 2010. Both calculations include green abscised preformed leaves to fully capture stand-scale response to heat and avoid potential bias from falsely disqualifying any normal (background) summer leaf abscission. The apparent contradiction between high neoformed leaf production and decreased end of season litter collection (g) is explained by low leaf mass in both preformed and neoformed leaves relative to average years (see fig. 13). Substantial LAI decreases are explained by significantly reduced areas of both preformed and neoformed leaves in 2010 (Fig. 10). Since neoformed leaves formed late in the season, the actual reduction in 'LAI-days' is expected to have been much greater than is apparent from this comparison of changes in cumulative end-of-season LAI.

Early season measurements of neoformed leaves (Ne leaves) showed significantly reduced photosynthetic capacity compared against normal leaves, with an average $A_{\text{max}}$ of 2.40 $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$. Although average Ne $A_{\text{max}}$ was not significantly different from Pe $A_{\text{max}}$, timing of leaf development (after heat stress) suggests that reduced photosynthetic capacity is attributable to different factors in Ne and Pe leaves. Both early leaf ontogenetic stage (Meir et al. 2002; Niinemets & Valladares 2004) and nutrient depletion (Field & Mooney 1983) could have produced reduced photosynthetic capacity in Ne leaves. Although % N was not determined for Ne leaves, prior work in the study region suggests that low Ne $A_{\text{max}}$ is better explained by sampling early in leaf ontogeny than by nutrient deficiency. Forests in the study region receive high rates of atmospheric N deposition (Ro and Vet, 2003), and previous work has shown that the growth of Sugar Maple at the study site is not N limited (Gradowski & Thomas 2006). Trees at the study site may be P limited (Gradowski & Thomas 2006) but liming to increase P availability
and the addition of P + K to soils has not been found to significantly impact $A_{\text{max}}$ in these trees (Gradowski & Thomas 2008). These results are consistent with other studies (Ellsworth & Reich 1992; Liu, et al. 1997), which have found only weak correlations between nutrient status and $A_{\text{max}}$ in Sugar Maple.

Data collected later in the growing season (July/August) showed that photosynthetic capacity in both preformed and neoformed leaves had recovered to values not significantly different from prior year measurements (5.27 and 5.97 µmol CO$_2$ m$^{-2}$s$^{-1}$, respectively). Neither leaf type was constrained by photoinhibition, suggesting that any high-temperature related damage to the photosynthetic apparatus was repaired by late summer. Note that interpretation of chlorophyll fluorescence measurements (see Fig. 12) was complicated by the absence of firm thresholds past which photodamage may be inferred. $F_v/F_m$ values for healthy plants have been reported as 0.832 ($\pm$ standard error of 0.004) for a diverse array of plant species (Bjorkman & Demmig 1987), and it has been asserted that $F_v/F_m$ values "close to 0.8" (Cavender-Barres & Bazzaz 2004:740) may be taken as a threshold below which any terrestrial plant may be considered stressed. However, previous authors have reported values of 0.776 in healthy mature Sugar Maples (Lee et al. 2003), values close to 0.832 in un-stressed shade leaves (Koniger et al. 2000) and large diurnal variation in $F_v/F_m$ in saplings (Zarco-Tejada et al. 2000). Values reported in this study are therefore considered to be within the normal range for the study species.

Similar recovery in photosynthetic capacity occurred in both leaf types despite significant differences in % N content and apparent chlorophyll concentration. Decreased absorbance in neoformed versus preformed leaves (see Fig. 15) between ~500 nm (upper
canopy) / ~490 nm (lower canopy) and ~690 nm (both upper and lower) indicate higher chlorophyll concentrations in preformed leaves (Verdebout et al. 1994; Penuelas & Filella 1998; Carter & Knapp 2001). This decrease in absorbance is consistent with observed reductions in reflectance from 500-660 nm reported by Baltzer and Thomas (2005) in Sugar Maple seedlings grown with enhanced nutrient supply, and with significant decreases in % N in neoformed versus preformed leaves (see Fig. 14). Comparable recovery of photosynthetic capacity in neoformed and preformed leaves despite significant differences in % N and chlorophyll content presents a puzzling question: why would trees invest heavily in early season preformed leaves if higher resource investment does not increase photosynthetic capacity? The answer may lie in N-saturation of Sugar Maple at the study site, which removes the assumption of resource scarcity under upon which allocative explanations are based. In an N-saturated system, high N levels in preformed leaves may constitute an unprofitable over-investment that is a byproduct of high N availability rather than an optimization strategy maximizing leaf function.

2.3.5 Age/Size Trends in Leaf Loss and Reflushing

Leaf loss and refushing showed strong differences in age/size response. Leaf loss was significantly lower in saplings than in all other trees (see Fig. 9; Table 3). Low levels of leaf loss in saplings are likely a result of their location in the more shaded understory, where both temperatures and light penetration are greatly reduced (Niinemets 2007). Lower neoformed leaf production in saplings could therefore be explained as the result of reduced damage within this ontogenetic class, but refushing vigor (indicated by the ratio
of neoformed leaves produced per preformed leaf lost, $N_L$) was also significantly lower in saplings (see Fig. 9).

The capacity to resprout (e.g., the degree to which trees are able to form new buds following damage should resource be available) is generally highest in saplings for temperate woody angiosperms (del Tredici 2001). As a result, lower reflushing vigor in saplings is best explained by resource constraints. Younger trees generally have smaller reserves of non-structural carbohydrates and consequently a reduced actual ability to resprout or reflush (Niinemets 2010). It is possible that the specific characteristics of high-temperature damage reduce the ability of saplings to recover relative to older trees (e.g. seedling resprouting in Sugar Maple has been found to be high following logging (Jacobs 1974) but low relative to older trees following ice storms (Duguay et al. 2001; del Tredici 2001); this difference could be explained by freeze-kill of shallow sapling roots during storms). However, lower damage (leaf loss) in saplings suggests that resource constraints rather than damage severity best explain low reflushing vigor.

High neoformed leaf production in mature canopy trees is consistent with large reserves on non-structural carbohydrates in these later ontogenetic stages (Niinemets 2010). Interpretation of the significant interaction term level×class (see Fig. 9) reveals Neo leaf production declined dramatically in the upper canopies of the largest trees. These large size class trees had an average dbh of 62.6 cm and were the largest individuals at the study site. Since the risk of xylem cavitation increases with hydraulic path length and gravitational potential (Domec et al. 2008), it is possible that decreased vascular connectivity following high temperature stress accounts for the absence of high neo leaf production at these (most optimal) canopy locations.
2.3.6 Optimization of Neoformed Leaf Production and Photosynthesis

Neoformed leaf production was concentrated in the upper canopy of mature trees (see Fig. 9), consistent with whole-tree photosynthetic optimization along canopy light gradients (Meir et al. 2002; Niinemets & Valladares 2004). However, the apparent chlorophyll content of neoformed leaves indicates a reduced capacity to optimize light use along vertical gradients compared to the first (preformed) flush. Significant differences in absorbance between preformed and neoformed leaves were greater in the lower than the upper canopy (see Fig. 16), with preformed leaves absorbing up to a maximum of 18% (lower) and 14% (upper) more light than neoformed leaves. Maximum differences occurred at 719 nm and 734 nm respectively, a region most sensitive to chlorophyll concentration (Curran et al. 1990). Differences in N content between neoformed and preformed leaves were less in the upper (0.279%) than the lower (0.428%) canopy, but larger disparities in the lower canopy were driven by high N content in preformed leaves.

Plants attempt to maximize whole-plant photosynthesis by allocating relatively more resources to optimally placed upper canopy leaves during reflushing (e.g. Hollinger 1996; Meir et al. 2002; Hikosaka 2005), and thus disparities in absorbance between canopy strata might be expected to result from increased N investment in the upper canopy during reflushing. However, data presented here suggest that greater differences between leaf types in the lower canopy are the result of increased investment in original lower canopy preformed leaves. Such investment may maximize the use of scarce light
resources in the lower canopy (Niinemets 2007), a strategy that would not be possible if resources were relatively more constrained during reflushing.

Although differences in neoformed leaf production between small, medium and large trees at lower and middle canopy levels were not found to be significant (Fig. 9; Table 3), increased mean leaf production in lower canopy levels in large trees is consistent with recent work on ontogenetic declines in intra-crown leaf area index (Nock et al. 2008). These authors found large ontogenetic declines in intra-crown LAI in Sugar Maple, with consequent increases in light penetration through the canopy in larger trees (e.g., roughly 4% of total sunlight for large size class trees). Based on these findings, it is possible that increased reflushing in the lower canopies of larger trees represents an attempt to utilize increased light penetration in lower canopy strata.

2.3.7 Neoformed versus Preformed Leaves: Acclimation to High Temperatures?

Gross leaf morphology (e.g., leaf shape) displayed characteristic variations between preformed and neoformed leaves that may indicate enhanced cooling properties in the latter leaf type. Although sinus angle was not measured in this study, neoformed leaves could reliably be identified in the field by more acute angles of secondary lobe divergence, thinner lobes, and a less compact structure (See Fig. 4, Fig. 5). Similar variations in leaf shape (less compact) have been linked to enhanced cooling properties in heat-response Pelargonium leaves (Nicotra et al. 2008), due to the creation of thinner boundary layers and consequently higher rates of leaf-air heat exchange. However, the evolutionary history of Sugar Maple suggests that gross morphological variation should be considered coincidental rather than adaptive with respect to cooling properties: since
neoformed leaves have evolved as a compensatory response to mechanical damage (insect defoliation; ice storms) rather than high temperature stress, there is no basis upon which to interpret morphological changes as driven by high-temperature acclimation.

No significant differences in calculated optical indices were observed between leaf types, although responses in relative concentrations of the major pigment groups examined in this study were expected based on theory. Carotenoids, particularly the xanthophylls, play a photoprotective role by dissipating excess light energy (Demmig-Adams et al. 1996; Penuelas & Filella 1998; Gitelson et al. 2002; Merzlyak et al. 2003), while evidence suggests that anthocyanins protect plants against photoinhibition (Neill & Gould 1999; Gitelson et al. 2001; Merzlyak et al. 2003; Merzlyak et al. 2008) and are formed in response to a variety of environmental stresses (Gitelson et al. 2001; Berg & Perkins 2005). Although relatively higher carotenoid concentrations (1CRI<sub>550</sub>; see table 2) in upper versus lower canopy leaves of both types could indicate increased photoprotective capacity in the upper canopy, these differences were not found to be significant across treatments. Carotenoids also absorb in the region between 490/500 nm and 690 nm and thus may have been present in higher concentrations in neoformed leaves (see Fig. 15), but their relative contribution to measured spectra is masked by chlorophyll absorbance (Gitelson et al. 2002). No significant differences were observed in relative anthocyanin concentrations. However, chlorophyll concentrations assessed using the mSR were also not significant, even though large significant differences were found between neoformed and preformed absorbance spectra in ranges associated with chlorophyll. Optical spectra were calculated from data that had been smoothed with a moving average, and as a result the lack of concordance between the mSR and visual
assessments of chlorophyll content using absorbance spectra is best explained by high instrument noise affecting the calculation of the spectral index. These results suggest that true differences in anthocyanin and carotenoid content were also masked. The very noisy nature of the data is shown in Fig. 16.

Differences in gas exchange parameters other than stomatal conductance (A_max and WUE) were not significant between leaf types (see Fig. 10 and 11). Significant differences in F_v/F_m between leaf types may indicate acclimation to higher temperatures in Neo leaves, but interpretation of these measurements is unclear. F_v/F_m ratios in both leaf types did not deviate from 'normal' values, and differences in F_o were not significant between treatments. This may be due to the small sample size for fluorescence measurements (n=3). However, F_v/F_m = (F_m-F_o)/F_m (see methods), and as a result an increase in F_o can yield lower values for this parameter. Calculation of F_v/F_m effectively normalizes F_o and may reveal significant trends, although the effect of changes in F_o on F_v/F_m are strong enough that changes in the efficiency of non-photochemical quenching can also be inferred directly from F_v/F_m measurements (Maxwell & Johnson 2000). Thus significantly lower F_v/F_m values for neoformed leaves (0.805 vs 0.822 in preformed leaves) may indicate an increased quenching capacity in neoformed leaves. This increased quenching capacity can arise from the disconnection of the large light harvesting complex (LHC) II antennae complex from PSII, or from reduced photochemical capacity (Briantias et al. 1996), and, if present, would indicate increased photoprotective capacity in neoformed leaves.
2.3.8 Sensitivity of Sugar Maple to Spring Heat Waves

Leaf fall during May was much higher in Sugar Maple (707. g/m²) than in other species (Fagus grandifolia Ehrh., Acer rubrum L., Betula cordifolia var. cordifolia Regel, Prunus serotina Ehrh.) collected following the May heat wave (0.74 g/m², cumulative). This represents 6.7% and 0.93% of total 2009 (see section 2.1.2) litter production in Sugar Maple and in all other collected species, respectively, indicating that larger summer litter collections for Sugar Maple were not simply the result of high species density at the study site. Two hypotheses were considered that could explain the more extreme response observed in Sugar Maple: i) timing of leaf development (phenology) in Sugar Maple caused the leaves of this species to be more vulnerable to heat in late May, and ii) reduced physiological adaptation to heat events in Sugar Maple caused trees to respond more negatively to heat.

Although recent work has begun to characterize ontogenetic changes in tree stress response (Niinemets 2010; Thomas et al. 2010; Voelker 2011), relatively little is known about such trends throughout leaf rather than tree development. It has been suggested that developing leaves may be more vulnerable to heat stress than mature leaves due to shifting priorities in resource allocation throughout leaf ontogeny (Coleman 1986), and maximum vulnerability to damage in intermediate leaf ontogenetic stages has in fact been shown with insect herbivory in Sugar Maple (Kolb & Teulon 1991). However, this allocative explanation for stress tolerance is more applicable to investments in chemical defense than to abiotic stress tolerance. Alternatively, leaf morphological changes throughout tree ontogeny have been implicated in ontogenetic changes in abiotic stress tolerance (Wieser et al. 2002), with younger trees exhibiting higher stress sensitivity.
Changes in the ratio of stomatal area to leaf area, internal leaf anatomy and conductance (Hanba et al. 2001; Oguchi et al. 2005) and concentrations of photoprotective pigments (but see Merzlyak et al. 2008) can also drive ontogenetic changes in abiotic stress tolerance. Such trends suggest that vulnerability to high temperature stress should change throughout leaf development, but this supposition has yet to be experimentally confirmed.

If Sugar Maple suffered severe summer leaf drop due to phenological vulnerability, tree species that did not exhibit leaf drop should tend to have leaves at a different ontogenetic stage than species that retained their leaves. Although canopy observations were not possible at the Megaplot, previous work has reported on bud-break and time to reflushing for Sugar Maple and several other hardwoods present at the study site. The hypothesis that leaf ontogenetic stage determined vulnerability to heat can therefore be evaluated by comparing the spring phenology of species at the megaplot that did or did not abscise leaves. Among temperate species, Sugar Maple has early or early/intermediate bud-break (Hunter & Lechowicz 1992; Richardson et al. 2006). Lechowicz (1984) found that full leaf expansion is reached later in *Acer saccharum* than in *Acer spicatum* Lamb. and *Prunus serotina* but much earlier than in *Acer rubrum*, *Prunus pensylvanica* Marsh., *Fagus grandifolia*, *Quercus rubra* L., *Betula alleghaniensis* Britt. and *Ostrya virginiana* (Mill.) K. Koch. *Fagus grandifolia* and *Quercus rubra* both reach full leaf expansion much later than all of these species (Lechowicz 1984). Species for which summer leaf drop was recorded (see fig. 6) thus do not exhibit similar spring phenology, suggesting that leaf ontogenetic stage did not determine whether leaves were abscised or retained. However, rejecting leaf ontogeny as the key criteria determining leaf
abscission does not imply that leaf ontogenetic stage (e.g., at partial expansion) during
the May heat wave did not affect vulnerability to high temperature stress.

Hypothesis ii) is better substantiated. It is clear that physiological differences
amongst tree species alter their damage tolerance to and ability to recovery from high
temperatures (Berry & Bjorkman 1980; Bassow et al. 1994). Strong general trends have
been found between plant growth environment and heat tolerance (Berry & Bjorkman
1980), with shade-tolerant species exhibiting less adaptation to high temperatures e.g.
(Dreyer et al. 2001) and possessing lower drought tolerance (Ninemets & Valladares
2006). As a result, shade tolerance was used as a proxy for physiological adaptation to
high temperature stress (table 4). A clear pattern is apparent from these results: shade
tolerance is seen to vary qualitatively with occurrence and severity of leaf drop. Although
sample bias may arise from differences in species densities in the Megaplot, contrasting
the responses of *F. americana* with *B. cordifolia* shows that shade tolerance, not species
density, determined whether leaves were collected. While there are two species that do
not fit the suggested trend (*P. serotina* and *O. virginiana*), these discrepancies can be
explained by species-specific ecophysiology. *P. serotina* has been shown to have a very
negative growth response to drought (Abrams et al. 1998) and exhibits ecotypic variation
in shade tolerance (Abrams et al. 1992), suggesting both that it is poorly adapted to heat
events and that accepted shade tolerance may not accurately represent the shade tolerance
of plot specimens. It is also noteworthy that in the group of species experiencing leaf
drop *P. serotina* exhibited the mildest response. Similarly, the shade-tolerant species *O.
virginiana* is drought resistant (Coder 1999b), suggesting it may have been
physiologically well adapted to withstand heat impacts.
Available evidence thus favors hypothesis ii): whether trees did or did not drop their leaves was related to interspecific differences in physiology rather than phenology. Contrasting the responses of *Q. rubra* and *F. grandifolia* highlights this point (Table. 4). Both reach full leaf expansion much later than all other species examined in this study, but only *F. grandifolia* exhibited leaf drop. This difference can be explained by physiology: *Q. rubra* has high optimum temperatures for photosynthesis (Dreyer et al. 2001) and has been shown to have physiological adaptations favoring survival from extreme heat (Huddle & Pallardy 1996). However, physiological tolerance changes with leaf ontogenetic stage and thus with phenology, and it is possible that Sugar Maple is especially physiologically vulnerable to heat stress when leaves are at an intermediate ontogenetic stage. Specific interactions between these factors and the consequences for tree response to heat events remain unexplored.

2.3.9 Leaf Abscission versus Sugar Maple Decline

Given the large body of work relating to Sugar Maple decline (for a review see McIlveen et al. 1986; Hendershot & Jones 1989; Houston 1999) it is likely that the observed summer 2010 leaf drop may be viewed as part of an ongoing decline process. Such a view confuses peripheral with central causes, and is not supported by the literature. Maple decline is a multi-year, multi-causal phenomenon (Hendershot & Jones 1989), whose symptoms (Roy et al. 2004) and age-distribution of effects (Auclair et al. 1996) are not consistent with heat impacts observed in this study. Most critically, past episodes of decline have not been associated with extreme high temperatures. Such climatic phenomena are fundamentally stochastic. They may contribute to decline by placing an
additional stress on Sugar Maples, but should be viewed as distinct from the suite of interacting processes such as acid rain (Duchesne et al. 2005), stand maturation (Auclair et al. 1996) or climatic warming (Griffin 1965) that have been proposed as underlying drivers of decline.

2.3.10 Underestimation of Reported Effects

It should be noted that results presented in this thesis tend to underestimate actual effects. Since standard leaf areas were calculated for late season leaves only, leaf loss has mostly been discussed here as g m\(^{-2}\) to avoid over representing summer leaf area losses (see methods). This figure grossly underestimates the loss of 'LAI-days' since leaf loss and leaf reflushing in the early summer reduced leaf cover for a substantial period versus 2009. Similarly, photosynthetic capacity (A\(_{\text{max}}\)) was assessed during two periods only (early season vs. late season), and so it is not possible to determine the rate of recovery following heat exposure. Our data suggest that photosynthetic capacity was severely reduced for longer than a month (June), suggesting large productivity losses throughout the summer season. Finally, the study site is located in an area that receives heavy N deposition (Ro and Vet, 2003), to the extent that trees are no longer N-limited (Gradowski & Thomas 2006). It is therefore reasonable to assume that trees subjected to heat impacts in areas not experiencing heavy N deposition would show reduced reflushing capacity and recovery of photosynthetic function relative to the study site.
2.3.11 Conclusion

High temperatures in late May 2010 were the primary driver for widespread leaf abscission and reduced photosynthetic capacity in Sugar Maple. Neither of these effects have been observed in prior years. Although neoformed leaf production partly compensated for leaf loss during the summer, both cumulative end-of-season litter production and LAI for Sugar Maple in 2010 were much lower than the only previous year for which data are available (reduced by 32.96% and 60.02%, respectively). Although similar reductions in litter production were observed in several associated species, on average impacts for these species were substantially lower than those observed in Sugar Maple. This increased vulnerability is most likely due to a low physiological tolerance to high temperatures in this shade-tolerant species. Strong ontogenetic trends in high temperature impacts were observed and related to differential stress exposure and resource availability in saplings versus mature trees. Photosynthetic capacity ($A_{\text{max}}$) was severely reduced in preformed leaves immediately following high temperatures in study trees, and in developing neoformed leaves. Although this parameter had recovered to normal levels in both leaf types by the end of the summer, photosynthetic capacity was reduced for a substantial portion of the growing season.

System productivity is necessarily related to the quantity of photosynthetic material present and the potential capacity of that material to sequester carbon through photosynthesis. As a result, the findings presented in this thesis are almost certain to correspond to a large productivity decline for Sugar Maple during the summer of 2010 at the study sites. While increased productivity in other species less affected by high May temperatures may have compensated for this decline, this effect is not investigated in the
present work. Observed responses of Sugar Maple to the May 2010 heat wave represent an important pathway for climate change impacts on temperate forests. Although large productivity declines following prolonged heat and drought events have previously been reported (Ciais et al. 2005; Allen et al. 2010), the current work documents large-scale and dramatic leaf loss and photosynthetic capacity declines from a short, landscape-level heat wave in the absence of any significant precipitation deficit. No similar phenomenon has been reported in the available literature.

An increase in the frequency and intensity of heat waves is likely as the climate continues to warm (Karl & Trenberth 2003; Meehl & Tebaldi 2004; IPCC 2007) and current models predict that this increase will be disproportionately large in the Northern Hemisphere (Meehl & Tebaldi 2004; WGI 2007). Shifts in spring phenology accompanying warming are predicted to cause earlier bud-break in Sugar Maple (Morin et al. 2009), resulting in an extended green canopy duration (Richardson et al. 2006). These shifts in phenology translate into an increased risk of vulnerability to biomass loss following heat waves, since leaf abscission may be a necessary response to high-temperature-induced evapotranspirative stress. Earlier flushing also increased the risks of frost damage (Norby et al. 2003), which may worsen heat sensitivity in Sugar Maple. Sugar Maple exhibits low adaptive differentiation to temperature across its range (Ledig & Korbobo 1983; Gunderson et al. 2000) and as a result it is unlikely that genetic variability in heat tolerance at the population level will allow the species to adapt to a high frequency heat wave climate. Repeated or more severe heat events could thus drive shifts in forest composition across the species’ range.
Although these outcomes are speculative, the results presented in this thesis suggest that the dramatic impacts of short-term acute heat events on temperate forests represent a previously unknown but potentially significant impact pathway of climate change. In the absence of a repeated heat event, retroactive studies (e.g. tree ring analysis) quantifying seasonal productivity losses and any impacts on community composition might be valuable in understanding how forests will function and change in a shifting global climate (see section 3.6).
2.4 Chapter Two Figures and Tables

Figure 1: Climate normals (1971-2000) for Haliburton town, ON. Data from Environment Canada (Environment Canada, n.d.). Shaded area indicated range of monthly average minimum and maximum temperatures for 2010.
Figure 2: a) May 2010 temperatures and b) precipitation (snow and rain) for Haliburton town, ON. Temperature is presented as daily ranges (bottom border: minima, top border: maxima). Arrows indicate days on which record-breaking temperatures were reported in Ontario. All data from Environment Canada (Environment Canada, n.d.).
Figure 3: a) View of Sugar-Maple-dominated deciduous canopy at Haliburton Forest in late May, following three days of record setting temperatures, and b) detail of Sugar Maple branch showing preformed leaves (dark green), scorched preformed leaves (brown) and developing neoformed leaves (light green). Photos courtesy of S.C. Thomas.
Figure 4: Typical preformed leaves collected at Kennisis Bridge July 30th-31st. Arrows indicate deformation resulting from loss of scorched leaf extremities. Note dark green color as compared to neoformed leaves (Fig. 5).
Figure 5: Typical neoformed leaves collected at Kennisis Bridge July 27th-30th. Note more diffuse (less compact) structure versus preformed leaves (Fig. 4).
Figure 6: Total May 2010 litter collection (dried mass) for the Haliburton megaplot. Only species in which May leaf fall was recorded are displayed. Bars represent one standard error of the mean.
Figure 7: Cumulative end-of-year litter production (dry mass) for Sugar Maple in the Haliburton megaplot, 2009 and 2010. Bars represent one standard error of the mean.
Figure 8: End-of-year leaf area index (LAI) for Sugar Maple in the Haliburton megaplot, 2009 and 2010. Bars show one standard error of the mean.
Figure 9: Results of calculated indices by canopy position and size class: a) average number of missing preformed leaves per internode (most recent years growth), b) ratio of neoformed leaf production to preformed leaf numbers, \( N_R \), c) ratio of neoformed leaf production to preformed leaf loss, \( N_L \), d) percent leaf mortality, e) percent leaf scorch, f) scorch score, \( S_S \). Bars show one standard error of the mean. Results of Tukey’s Honestly Significant Difference post-hoc test (see section 2.1.9) are presented in Table 4.
Figure 10: Maximum photosynthetic rate by year and leaf type. Measurements with different letters and significantly different at $p = 0.05$. P-e and N-e are early season measurements on preformed and neoformed leaves, respectively, while P and N are late summer measurements on both leaf types. Bars represent one standard error of the mean. Dotted line shows the 2002-2008 average.
Figure 11: Water use efficiency and stomatal conductance by year and leaf type. Measurements with different letters are significantly different at $p = 0.05$. P-e and N-e are early summer measurements on preformed and neoformed leaves, respectively, while P and N are late summer measurements on both leaf types. Bars represent one standard error of the mean. In both panes, the dotted line indicated the average for pre-2010 leaves (standard year leaves).
Figure 12: Fv/Fm values by canopy position. Different letters indicate significance at $p = 0.05$. Bars represent one standard error of the mean.
Figure 13: Morphological measurements by year and canopy positions. Within each figure measurements with different letters are significantly different from each other. Dashed line shows average values for 2002-2006 for high canopy samples; dashed and dotted line shows these averages for low canopy samples. Where high and low canopy positions did not display significantly different measurements only one line (dashed) is displayed. Bars show one standard error of the mean. Pre and Neo indicate preformed and neoformed leaf types, respectively.
Figure 14: Leaf carbon and nitrogen at high and low canopy levels. Different letters indicate significant difference at $p = 0.05$. Bars represent one standard error of the mean.
Figure 15: Absorbance, reflectance and transmittance spectra from neoformed and preformed leaves gathered from two canopy positions. Shaded areas indicate significant differences at $p=0.05$ (grey shading when preformed values exceed neoformed; line shading when neoformed values exceed preformed).
Figure 16: Percent differences in absorbance between leaf types obtained by subtracting neoformed from preformed absorbance spectra. Subtractions of this type are described by Carter and Knapp (2001), and allow easy visual identification of differences between spectra near 700 nm that may be masked by the steep slope of the "red edge" in untransformed data. Black lines is a lowess curve trendline (f=0.1), light grey shows actual variation in the data, and dark grey areas under the curve indicate significance at p=0.005. Note high levels of noise above 740 nm.
Table 1: Hill’s Criteria modified for complex ecological phenomena. Modified from Plowright et al (2008).

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Explanation</th>
<th>Criterion</th>
<th>Explanation</th>
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<tr>
<td>Strength of Association</td>
<td>Casual factor must be correlated with phenomenon and have explanatory power that may be assessed statistically</td>
<td>Consistency:</td>
<td>Casual factor must be associated with phenomenon repeatedly across time and space</td>
</tr>
<tr>
<td>Plausibility:</td>
<td>Casual explanation must make ecological sense</td>
<td>Coherence:</td>
<td>Casual explanation should be consistent with current body of knowledge</td>
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<td>Experimental Evidence:</td>
<td>Experimental manipulation of the causal variable should change the outcome</td>
<td>Analogy:</td>
<td>The casual hypothesis under consideration may be compared to an analogous relationship demonstrated in another system</td>
</tr>
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<td>Specificity:</td>
<td>The causal factor should be absent when the phenomenon is absent</td>
<td>Temporality:</td>
<td>The casual factor must precede the phenomenon in time</td>
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<tr>
<td>Biologic Gradient:</td>
<td>The causal factor should show a 'dose-response' relationship with the phenomenon</td>
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Table 2: Mean values for spectral pigment indices ± one standard error. Sample size is indicated as n=(sample size for mSR)/(sample size for ARI and $^{1}$CRI$_{550}$). No significant treatment effects (height or leaf type) were found at Pr(>F)>0.05. Note that indices are unitless.

<table>
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<th>Lower canopy</th>
<th>Lower canopy</th>
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<td>Pre leaves</td>
<td>Neo leaves</td>
<td>Pre leaves</td>
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<td>mSR</td>
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<td>1.843 ±0.650</td>
<td>0.991 ±0.562</td>
<td>1.212 ±0.374</td>
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<td>ARI</td>
<td>1.799 ±0.259</td>
<td>1.755 ±0.265</td>
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<td>$^{1}$CRI$_{550}$</td>
<td>7.673 ±1.463</td>
<td>7.408 ±1.331</td>
<td>5.703 ±0.998</td>
<td>5.904 ±0.901</td>
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Table 3: Initial MANOVA results for all variables. Non-significant variables were not considered for further analysis (see methods). Reported here are F values and significance for a linear model of the form: `gen=aov(cbind(missing,leaf.mort,scorch.score,scorch.percent,sqrt(NperLost),sqrt(neo.ratio))~class*level,data=large)`. Significance codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.

<table>
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<td>2.822*</td>
<td>2.565.</td>
<td>23.497***</td>
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<td>15.810***</td>
<td>8.706***</td>
<td>0.936</td>
<td>0.053</td>
<td>26.564***</td>
<td>39.360***</td>
</tr>
<tr>
<td>class×level</td>
<td>1.141</td>
<td>0.927</td>
<td>0.590</td>
<td>0.662</td>
<td>3.150**</td>
<td>4.055***</td>
</tr>
</tbody>
</table>
Table 4: Results of Tukey’s HSD (p adj) by response variable for multiple linear models of the form (variable)~class*level for leaf count data collected at Kennisis Bridge. Data is displayed in Fig.7. With 4 levels of predictor ‘class’ and 3 levels of predictor ‘level’ interpretation of differences between interaction terms is highly complex and prone to misinterpretation. Consequently differences between interaction terms are not reported. Size classes are indicates as V (saplings), S (small mature trees), M (mid-sized mature trees) and L (very large mature trees). significance codes: 0 ‘***’, 0.001 ‘**’, 0.01 ‘*’, 0.05 ‘.’; bold results are significant at p = 0.05.

<table>
<thead>
<tr>
<th>Class</th>
<th>M-L</th>
<th>0.9978</th>
<th>0.9999</th>
<th>0.9982</th>
<th>0.2599</th>
<th>0.3561</th>
<th>0.3088</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-L</td>
<td>0.9991</td>
<td>0.9945</td>
<td>0.9999</td>
<td>0.9999</td>
<td>0.9233</td>
<td>0.9994</td>
<td></td>
</tr>
<tr>
<td>V-L</td>
<td><strong>0.0015</strong></td>
<td><strong>0.0074</strong></td>
<td>0.1494</td>
<td>0.8740</td>
<td>&lt;0.0001***</td>
<td>&lt;0.0001***</td>
<td></td>
</tr>
<tr>
<td>S-M</td>
<td>0.9902</td>
<td>0.9910</td>
<td>0.9994</td>
<td>0.3063</td>
<td>0.8024</td>
<td>0.4465</td>
<td></td>
</tr>
<tr>
<td>V-M</td>
<td><strong>0.0034</strong></td>
<td><strong>0.0105</strong></td>
<td>0.1068</td>
<td>0.0557</td>
<td>&lt;0.0001***</td>
<td>&lt;0.0001***</td>
<td></td>
</tr>
<tr>
<td>V-S</td>
<td><strong>0.0026</strong></td>
<td><strong>0.0077</strong></td>
<td>0.1854</td>
<td>0.9220</td>
<td>&lt;0.0001***</td>
<td>&lt;0.0001***</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Level</th>
<th>M-L</th>
<th>0.0428</th>
<th>0.0717</th>
<th>0.9948</th>
<th>0.6800</th>
<th>0.14112</th>
<th>0.0100.</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-L</td>
<td>&lt;0.0001**</td>
<td>&lt;0.0001***</td>
<td>0.4643</td>
<td>0.9822</td>
<td>&lt;0.0001***</td>
<td>&lt;0.0001***</td>
<td></td>
</tr>
<tr>
<td>T-M</td>
<td><strong>0.0007</strong>***</td>
<td><strong>0.0240.</strong></td>
<td>0.4083</td>
<td>0.5670</td>
<td>&lt;0.0001***</td>
<td>&lt;0.0001***</td>
<td></td>
</tr>
</tbody>
</table>
Table 5: Shade tolerance and approximate southern range limits for common deciduous hardwoods at the study site. All data from Burns and Honkale (1990). Where applicable early summer leaf drop (May and June collections) is reported in brackets in the column ‘Leaf Drop?’ as a percentage of total 2009 collection, the only prior year for which complete data is available for this set of species. Note that since litter production is known to have been reduced in at least one species (Sugar Maple) in 2010 versus 2009 these figures are expected to under represent the magnitude of observed effects. Species names in bold indicate outliers (see section 2.3.8).

<table>
<thead>
<tr>
<th>Species</th>
<th>Leaf Drop? (g m⁻²)</th>
<th>2009 plot stem count</th>
<th>S. Range Limit (°N)</th>
<th>Shade Tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acer saccharum</em></td>
<td>YES (6.67)</td>
<td>2623</td>
<td>35</td>
<td>very tolerant</td>
</tr>
<tr>
<td><em>Acer rubrum</em></td>
<td>YES (2.21)</td>
<td>618</td>
<td>25</td>
<td>moderate / tolerant</td>
</tr>
<tr>
<td><em>Prunus serotina</em></td>
<td>YES (0.31)</td>
<td>74</td>
<td>15</td>
<td>intolerant</td>
</tr>
<tr>
<td><em>Fagus grandifolia</em></td>
<td>YES (0.57)</td>
<td>654</td>
<td>30</td>
<td>very tolerant</td>
</tr>
<tr>
<td><em>Betula cordifolia</em></td>
<td>YES (0.63)</td>
<td>176</td>
<td>40</td>
<td>shade tolerant</td>
</tr>
<tr>
<td><em>Quercus rubra</em></td>
<td>NO</td>
<td>76</td>
<td>30</td>
<td>intermediate</td>
</tr>
<tr>
<td><em>Ostrya virginiana</em></td>
<td>NO</td>
<td>43</td>
<td>30</td>
<td>shade tolerant</td>
</tr>
<tr>
<td><em>Fraxinus americana</em></td>
<td>NO</td>
<td>106</td>
<td>30</td>
<td>intolerant</td>
</tr>
<tr>
<td><em>Betula alleghaniensis</em></td>
<td>NO</td>
<td>163</td>
<td>35</td>
<td>intermediate tolerance</td>
</tr>
</tbody>
</table>
Table 6: Average neoformed leaf production per internode by canopy position and tree size (± one standard error of the mean). The effects of both canopy level and tree size class are significant.

<table>
<thead>
<tr>
<th></th>
<th>Saplings</th>
<th>Small</th>
<th>Medium</th>
<th>Large</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High Canopy</strong></td>
<td>1.58 ±0.53</td>
<td>20.94 ±2.53</td>
<td>23.29 ±1.60</td>
<td>12.38 ±1.28</td>
</tr>
<tr>
<td><strong>Mid-Canopy</strong></td>
<td>0.55 ±0.21</td>
<td>4.39 ±1.10</td>
<td>5.95 ±1.10</td>
<td>7.42 ±1.51</td>
</tr>
<tr>
<td><strong>Low Canopy</strong></td>
<td>0.23 ±0.16</td>
<td>2.24 ±0.55</td>
<td>2.10 ±0.52</td>
<td>2.56 ±0.73</td>
</tr>
</tbody>
</table>
Table 7: Results ($p$-values) of analysis of covariance for gas-exchange measurements and related parameters. Results which are significant at $p=0.005$ are in bold. See sections 2.1.3, 2.1.6, 2.1.7 and 2.1.9 for a description of these metrics and of analytical procedure.

<table>
<thead>
<tr>
<th></th>
<th>Leaf C</th>
<th>Leaf N</th>
<th>Leaf Mass</th>
<th>LMA</th>
<th>LMA$_{lamina}$</th>
<th>WUE</th>
<th>$A_{max}$</th>
<th>$g_s$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf Type</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.6325</td>
<td>0.0061</td>
<td>0.0428</td>
</tr>
<tr>
<td>Canopy Strata</td>
<td>0.0096</td>
<td>0.3434</td>
<td>0.1423</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.1539</td>
<td>0.7034</td>
<td>0.0641</td>
</tr>
<tr>
<td>Leaf Type × Canopy Strata</td>
<td>0.3353</td>
<td>0.8233</td>
<td>0.9557</td>
<td>0.2950</td>
<td>0.3089</td>
<td>0.6361</td>
<td>0.7525</td>
<td>0.3003</td>
</tr>
</tbody>
</table>

$n$ 82 82 169 73 73 77 181 192
Chapter 3: Conclusion and Implications

3.1 Introduction

The results presented in this thesis represent the first investigation of the effects of a short duration heat wave across multiple ontogenetic stages in a temperate deciduous tree. Key findings and future research directions are summarized in Fig. 17. Since heat waves are predicted to increase in frequency, duration and intensity (Karl & Trenberth 2003; Meehl & Tebaldi 2004; IPCC 2007), the effects associated with high temperature stress are expected to be of increasing importance. From this perspective, this chapter attempts to discuss the broader implications of observed trends for carbon cycling, forest management and ecosystem modeling, as well as to revisit key themes in light of the material presented in Ch. 2.

3.2 Event-Driven Research, Revisited

Environmental phenomena such as frost events, droughts or heat waves may occur infrequently, and may have impacts at very large spatial scales. Because of their magnitude and scale, experimentally replicating these events may be impossible (Hilborn & Ludwig 1993; Plowright et al. 2008). Scientific inquiry into the effects of these phenomena on forests thus requires data collection in the field during and after these events; such 'event-driven' research has been used successfully to explore the impacts of ice storms (e.g. Hooper et al. 2001; Brommit et al. 2004), droughts (e.g. Breshears et al. 2005; Carnicer et al. 2011) and frosts (e.g. Smith and Cannel 1986) on temperate forests. In addition to allowing the quantitative study of non replicable large-scale environmental
phenomena, event-driven research is highly responsive to current issues, allows the rapid evaluation of climate impacts, does not require the high set-up costs associated with large-scale experiments, and includes all relevant factors involved in producing observed effects.

However, event-driven research also suffers from severe drawbacks as compared to planned experiments. A lack of knowledge, limited sampling times and logistical difficulties in mobilizing resources may complicate the collection of useful data, and where the research topic is relatively unstudied it may be difficult to know which aspects of the observed phenomena merit the most attention. More critically, firmly establishing causality between environmental phenomena and observed effects may be problematic, since key elements of traditional reductionist hypothesis testing (experimental control, replications and randomization) may be impossible to achieve in the field (Hilborn & Ludwig 1993; Plowright et al. 2008). Consequently causality in event-driven research is usually inferred, although options exist to more rigorously evaluate causality using qualitative rubrics such as Hill's Criteria (Hill 1965; Plowright et al. 2008; section 1.0.1). Yet even where causality can be established, partitioning the effects of multiple interacting factors may be impossible without the incorporation of experimental controls. In addition, the unique climatic history and site characteristics of any study site limit the degree to which the results of an event-driven research effort can be generalized. Experimental inquiry into specific aspects of a studied phenomenon may thus be necessary to address unresolved questions following an event-driven research effort.

While the research effort summarized in this thesis demonstrates the utility of event-driven inquiry, it also suffers from some of the aforementioned drawbacks.
Intensive data collection throughout the summer of 2010 created an opportunity to investigate an important and heretofore neglected climate change impact pathway on temperate forests, but some key questions remain unanswered (section 3.7). Improved data collection during 2010 could potentially have helped answer these questions, and would certainly have provided a more in-depth picture of Sugar Maple damage and recovery following high temperatures. For example, the collection of $A_{\text{max}}$ survey measurement on a smaller pool of individuals at regular intervals throughout the summer would have given a more accurate picture of the dynamics of tree recovery from heat stress, while fluorescence quenching data gathered in early June could have shed light on the relative importance of heat and water stress in producing observed leaf damage. Some other aspects of the work (e.g. sampling preformed leaf loss and neoformed leaf production across both vertical and ontogenetic gradients) could have been reduced in scope (for instance, by only having two size classes for mature trees).

These misapplications of sampling effort demonstrate a key problem in any research effort: until data have been collected and analyzed, it is difficult to tell which trends are important. This can become a major problem in event-driven research, where a limited window for sampling means that inefficient data collection wastes a rare opportunity to study the phenomenon of interest. However, improved sampling strategies may help rectify this problem. Similar to the principles of adaptive environmental management (Walters 1986), a recursive, adaptive sampling strategy could help focus data collection on the most important trends. At it's most basic, such a strategy would emphasize the need to conduct preliminary data analysis during the sampling season, identifying the most important trends and focusing subsequent sampling effort on their
exploration. Initial broadly targeted survey data could be used to determine which areas merited in-depth inquiry, with an initial high frequency of self-evaluation (e.g. daily or weekly) reducing substantially throughout the sampling window (e.g. to monthly by the end of the summer) as the research effort became more 'committed' to certain aspects of the phenomenon of interest. Of course, such a strategy presupposes sufficient prior knowledge to efficiently analyze data and distinguish noteworthy trends.

3.3 The Nature of Observed High Temperature Stress

Observed high temperature impacts constituted a substantial stress on Sugar Maple at the study site. Leaf loss, productivity declines, possible decreases in vascular connectivity in large trees and large resource investments in neoformed leaf production are expected to have significantly altered ecosystem functioning in 2010 compared to prior years. However, the stress exerted by high May temperatures was not catastrophic. Sugar Maples appeared to recover in the months following high temperature stress, and no mortality or crown dieback was observed during a visit to the Kennisis Bridge study site during 2011. Yet stresses within any ecosystem have the potential to interact dynamically with other factors, biotic and abiotic, that may negatively influence ecosystem functioning. This has been demonstrated extremely clearly in the specific case of Sugar Maple, where the large literature on Sugar Maple Decline (see section 1.7) has often focused on the potential for multiple interacting stress factors to cause mortality.

Given the interacting nature of stresses in forest ecosystems, the potential role of high temperature stress on temperate forests is best understood through the theoretical lens of the Multiple Stress Hypothesis (MSH). The MSH holds that tree mortality is the
product of multiple factors, classed as predisposing, inciting and contributing (Hendershot & Jones 1989). Predisposing factors are the stress agents that weaken healthy trees, opening the door to further damage. Inciting factors are secondary stress agents that contribute to the further deterioration of tree health, while contributing factors are those stresses that ultimately cause mortality. For example, stand overmaturity (predisposing factor) could produce a large population of senescent trees vulnerable to attack by insect pests (inciting factor), which might then be unable to resist a fungal outbreak causing mortality (contributing factor).

The effects of high May temperatures reported in this thesis suggest that short-term, acute heat waves have the potential to act as predisposing or inciting factors only. Sampled trees suffered from productivity losses and the necessity to expend resources during leaf neoformation, but did not appear to sustain direct damage. A possible exception is the suggested loss of vascular connectivity in the upper crowns of large size class trees, but both known ontogenetic trends in non-structural carbohydrate reserves (Niinemets 2010) and high refushing ability lower in the crown (Fig. 9b) argue that these trees are most able to recover from this damage. In all other cases profuse refushing suggests that leaf abscission on high temperature days prevented irreversible xylem cavitation (Fig. 9b&c). In the absence of direct damage pathways heat waves such as that reported in this thesis should be unable to cause tree mortality, and thus should not be able to act as contributing factors to tree mortality. However, productivity losses / resource expenditures are predicted to weaken trees, making them less able to resist further stresses. For study trees, high temperatures during May 2010 are best
characterized as a predisposing stress, since no other pre-existing stress factors were evident.

Characterizing short-duration heat waves as predisposing or inciting stresses informs an understanding of their potential role as the climate continues to warm. An increased frequency of heat waves means that trees such as Sugar Maple which are poorly adapted to the effects of high temperatures will increasingly suffer from another stress factor that could contribute to mortality. As has been seen with the phenomenon of Sugar Maple decline, the combined effects of climatic with other stresses can have extremely large impacts on forest ecosystems and related human economic systems (Hendershot and Jones 1989; section 1.4). The results presented in this thesis must therefore be considered not only in terms of their direct impacts on Sugar Maple, but also by their potential to interact with other factors, indirectly producing a much larger effect on tree health. Of course, specific outcomes cannot be predicted, but as high temperature events become more frequent, more intense or of greater duration (IPCC 2007) the threat they pose to forest health (and particularly to Sugar Maple) will extend beyond simple reductions in productivity.

3.4 Impacts on Carbon Cycling

The predicted impacts of global warming on terrestrial carbon cycling are complex, and involve multiple interacting feedback processes (for a review see Luo 2007). The impacts of warming on carbon sequestration in temperate forest ecosystems, particularly during the spring months, are not yet fully understood. Previous work has suggested that earlier bud break and leaf out (spring 'greening') associated with climate change (IPCC 2007;
Borchert et al. 2005) will result in increased carbon sequestration during the spring months (Arain et al. 2002; Angert et al. 2005; Luo 2007; Welp et al. 2007). However, these gains could be partially offset by productivity losses resulting from an increased risk of damage from late frosts (e.g. Smith & Cannel 1986; Saxe et al. 2001) and recent work has found divergent responses to spring warming in dominant conifer species in the Canadian Boreal (Nelson and Thomas, in review). The results presented in this thesis further challenge the assumption that spring warming will increase productivity.

Both \( A_{\text{max}} \) and LAI are closely related to carbon uptake (Street et al. 2007; Thomas 2010). Consequently, documented reductions in \( A_{\text{max}} \) of \( \sim 64\% \) throughout the late spring and summer, combined with a \( 32.96\% \) reduction in leaf litter production (g m\(^{-2}\)) and a \( 60.02\% \) reduction in LAI (Sugar Maples only) for 2010 are expected to have been matched by large productivity declines in affected trees. Preliminary measurements of carbon flux at a Sugar Maple-dominated site at Haliburton forest suggest that these reductions affected carbon sequestration throughout the growing season in 2010, creating a slight C source at the landscape scale in late summer (Thomas et al. 2011). These results suggest a novel mechanism (spring heat waves) by which anthropogenic global warming can alter seasonal carbon cycling in temperate forest ecosystems.

However, the potential effect of heat waves on temperate forests goes beyond direct impacts on productivity. Interactions with other stress factors may work to magnify or reduce the impacts of high temperatures. It has been argued in section 3.3 that the observed late May heat wave is best characterized as a predisposing or inciting stress. The snowballing effects of such stresses can be profound: for example, Galinski and Witowski (1995) document large C release from a Polish forest subject to sanitation cuts.
following a succession of insect infestations. Initial insect attacks (predisposing factors) did not cause mortality, but did precipitate a chain of events leading to a massive alteration to landscape-level C cycling. Although such ramifications remain speculative in the case of heat wave impacts on Sugar Maple, the scale and severity of Sugar Maple decline, itself most plausibly a product of multiple interacting stress factors (see section 1.7.4), argues for an awareness of the potential indirect effects of high spring temperatures.

Downstream effects on carbon cycling may also act to modify the net impact of heat waves on forested ecosystems. Alterations to disturbance regimes in forest ecosystems affect human economic as well as natural ecosystem carbon pools (Apps & Price 1995). Consequently, understanding net effects of global C cycling requires a consideration of the socioeconomic context within which forests are, in the 21st century, embedded. Downstream carbon pools that are affected by alterations to natural forest ecosystems can be broadly classed as forest products, fossil fuels and landfills (Apps & Price 1995), carbon storage in all of which could potentially be affected by heat wave induced productivity losses in temperate forests. For example, high temperature-induced crown dieback in a (hypothetical) sugarbush affected by a late spring heat wave would be expected to reduce sap volume and quality (Houston et al. 1990) and thus maple syrup production. Input costs (transportation, refining, etc.) should scale with syrup production, and thus initial reductions in C sequestration due to reduced ecosystem productivity could be partially offset by corresponding reductions in fossil fuel use. Although such effects are not closely related to the material presented in this thesis, an awareness of their potential to amplify or reduce initial alterations to C storage is highly desirable for
researchers interested in understanding how climatic phenomena impact C cycling at large (e.g., provincial, national or global) spatial scales.

**3.5 Implications for Process-Based Modeling**

Process-based models of ecological systems are important tools for testing theories about ecosystem functioning, and for predicting climate change impacts. These models are generally highly sophisticated, and when current substantial effort may go towards refining and improving their predictive capacity. Improvements aim to more accurately simulate ecosystem processes, for example by incorporating stratified layers of sun and shade leaves rather than assuming a single homogenous ('big leaf') leaf layer (e.g. Liu et al. 1997) when modeling gas exchange in forests. Although models may differ substantially in their required inputs, leaf-level biochemical and structural characteristics are often integral to simulating ecosystem processes (e.g. net primary productivity) at landscape scales. For example, the Canadian Boreal Ecosystem Processes model (BEPS) requires leaf nitrogen content to calculate mesophyll conductance (Liu et al. 1997), while the biogeochemical simulator BIOME-BGC incorporates leaf morphological measurements and C:N ratios into its calculations of leaf area index (White et al. 2000).

The results presented in this thesis suggest that spring heat waves can substantially reduce productivity in temperate trees, that this reduction is visible at the leaf level, and that the effect on productivity throughout the growing season is some combination of periods of damage, recovery and normal function. Preliminary remote sensing work (see Fig. 18) indicates that these effects are visible at the landscape scale. Some of this change in productivity could be captured by simple reparameterization of
model inputs (e.g. attempting to calculate average input values for leaf level processes in heat wave years), but this approach would fail to capture the temporal dynamics of damage and recovery. Such temporal variation is of central importance where variation in other climatic parameters (e.g. precipitation) exhibits seasonality within the model and exerts a strong effect on productivity. Substantial changes in leaf area index and photosynthetic capacity throughout the summer of 2010 thus suggest that any model that assumes leaf characteristics are constant once leaves are developed risks severely misrepresenting actual ecosystem processes. Incorporating functionality which allows leaf characteristics to vary, and perhaps allows multiple leaf types with distinct biochemical and morphological characteristics to occur simultaneously, could help process-based models capture the effects of heat waves on temperature forest ecosystems.

3.6 Implications for Sugar Maple Management

The results presented in this thesis suggest that even very short heat waves have the capacity to significantly alter forest form and functioning. Impacts are expected to be exacerbated when other stress factors are present. The ability of forest managers to reduce heat wave impacts is extremely limited, since as an intrinsic part of global-scale warming trends high temperature events are not easily prevented or ameliorated. Management prescriptions must therefore focus on adapting to a changing climate rather than maintaining the status quo. Sugar Maple is a late seral stage, shade-tolerant species (Godman et al. 1990), with a relatively poor ability to tolerate heat stress (see section 1.9.1). Available work indicates that the species does not exhibit much genetic differentiation throughout its range, and has a limited capacity to adapt to a warmer
temperature regime (Ledig & Korbobo 1983; Gunderson et al. 2000). The ability of forest managers to select for heat-tolerant genotypes is therefore likely to be constrained, and should heat wave frequency or intensity increase beyond (currently unknown) tolerance thresholds it may be necessary to substantially reduce the economic role of the species in affected areas. Fortunately, the highly heterogeneous responses of sampled tree species to late May temperatures documented in this study suggest that diversification of managed species represents a viable adaptive strategy to cope with heat wave impacts.

However, some suggestions can be made for ameliorating high temperature impacts on sugarbushes. Worth an estimated $15 million annually in Ontario alone (OMAFRA 2011), the high value and small size of sugarbushes may allow management options that are not viable for larger harvested forests. Careful site selection and watering during high temperature events are possible options for increasing sugarbush resilience to heat waves. The effects of high temperatures tend to be exacerbated by evapotranspirative stress and induced drought (see section 1.1). Consequently, watering both seedlings and mature trees on days during which extreme temperatures occur may help to reduce high temperature impacts (Helgerson 1990; Coder 1999a). In practice this may only be feasible where sugarbushes are located near an abundant water source such as a lake or stream. In addition to locating newly developed sugarbushes by water sources, location in cooler, moister and more shaded sites may help reduce the impacts of high temperatures on cultivated Sugar Maple. However, choosing such sites may result in reduced productivity (Houston et al. 1990), and it may therefore prove impossible to select sites that are both resilient to high temperatures and economically viable.
3.7 Conclusion and Future Directions

This thesis describes a previously little explored climate change impact pathway on temperate forests. The results of an event-driven research effort focused on the effects of an unseasonal spring heat wave on Sugar Maple suggest that such events have substantial negative impacts on productivity in temperate species that are not adapted to withstand high temperature stress. Preliminary measurements of carbon flux (Thomas et al. 2011) from a research site located in the study region (Haliburton Forest) indicate that these effects may be visible at the landscape scale, and persist throughout the growing season, while preliminary remote sensing work (Fig. 18) suggests that a very large geographic area can be affected. These findings challenge widely held assumptions about the beneficial effects of spring warming, and argue for the incorporation of disturbance-driven temporal variation in leaf level parameters in process-based ecosystem models. Decreases in productivity caused by heat waves may also negatively impact managed Sugar Maple stands (harvested timber and Maple Syrup production), but due to the scale of these impacts, and their highly non-preventable nature, a management focus on adaptation rather than prevention is required.

As an event-driven research effort, this study suffered from drawbacks associated with limited preparation and sampling time. Sampling effort was concentrated too heavily in some areas, and some important avenues of inquiry were not recognized. Consequently, the results presented here suggest numerous additional research questions (see Fig. 17). Firstly, it was not possible to fully partition the effects of species physiological vulnerability and leaf ontogenetic stage in determining observed responses of Sugar Maple to high temperatures in late May. Strong qualitative trends in
ecophysiological adaptation in affected versus unaffected tree species at one study site (Megaplot) argue that physiological vulnerability was of primary importance in determining tree responses to high temperature stress (see 2.3.8), but leaf ontogenetic stage may still have contributed to tree response. Investigating leaf ontogenetic trends in high temperature vulnerability could be an important addition to our understanding of the importance of seasonality in climate change impacts, and could help explain the severity of high temperature responses observed in 2010. While warming studies with saplings could help explore this question, interpretation of results would be complicated by the unknown effects of tree ontogenetic stage. Ideally, canopy heating experiments in mature trees throughout the spring and early summer should be utilized to explore possible leaf ontogenetic effects.

Secondly, it was not possible to determine the relative contributions of high temperatures and induced water stress to observed leaf loss and leaf damage. An attempt was made to address this question using measurements of chlorophyll $\alpha$ fluorescence, but poor data collection prevented an in-depth analysis. A properly designed quenching analysis conducted on heated leaves (e.g., in combination with canopy heating experiments) could be highly informative in this regard. Furthermore, if neoformed leaf production could be experimentally induced by heating, fluorescence measurements could also be used in combination with temperature response curves of photosynthesis, analyses of heat shock protein formation, and spectral analyses of pigment concentrations to investigate biochemical adaptation of heat-response leaves to higher temperatures.

Thirdly, the effects of high May temperatures on carbon cycling remain largely unexplored. While retroactive studies examining incremental annual growth could be
used to explore the net effects of high temperatures on productivity in 2010, landscape-level measurements of carbon flux are required to reveal the effects of the May heat wave on ecosystem productivity throughout the growing season. In particular, the temporal dynamics of high temperature 'damage' (productivity declines) and recovery could be assessed using this method. If used in combination with an inventory-based analysis of net annual productivity (e.g. incremental annual growth across multiple species), flux measurements could be used to explore how species diversity buffers climate change impacts at the landscape scale.

Finally, due to the predicted increase in the frequency, duration or intensity of heat waves (WGI 2007), increasing our capacity to predict high temperature impacts on forest processes is of clear importance. Developing functionality to incorporate heat wave occurrence, impacts and recovery into process-based ecosystem models will help predict climate change impacts on forest ecosystems and associated human economic endeavors, as well as explore how this feedback mechanism could affect future rates of climate change. In addition to modeling efforts, establishing ecophysiological guidelines to determine species-specific sensitivity to high temperature stress, as well as thresholds for temperature tolerance, will further our capacity for resilient adaptation to climate change.
3.8 Chapter Three Figures and Tables

<table>
<thead>
<tr>
<th>Key Findings</th>
<th>Future Directions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reduced Productivity</strong></td>
<td>Can guidelines be established for predicting stress sensitivity in temperate tree species?</td>
</tr>
<tr>
<td>• Sensitivity of Sugar Maple</td>
<td>Did leaf ontogenetic stage increase vulnerability?</td>
</tr>
<tr>
<td>• Summer leaf loss</td>
<td>How do high temperatures and induced water stress interact to cause leaf loss?</td>
</tr>
<tr>
<td>• $A_{\text{max}}$ declines in early summer</td>
<td>What was the effect on carbon cycling and sequestration during 2010?</td>
</tr>
<tr>
<td>• Reduced end-of-year leaf production in 2010</td>
<td></td>
</tr>
<tr>
<td><strong>Ontogenetic Trends</strong></td>
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<tr>
<td>• Low flushing vigor in saplings</td>
<td></td>
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<tr>
<td>• Cavitation in upper canopy of older trees (suggested)</td>
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</tr>
<tr>
<td><strong>Characteristics of Neo Leaves</strong></td>
<td></td>
</tr>
<tr>
<td>• High conductance</td>
<td>Did Neo leaves have modified concentrations of photoprotective pigments?</td>
</tr>
<tr>
<td>• Low N concentrations</td>
<td>Do Neo leaves have an altered temperature dependence of photosynthesis?</td>
</tr>
<tr>
<td>• Less compact shape</td>
<td></td>
</tr>
</tbody>
</table>

Figure 17: Key findings and suggestions for future research.
## References


Figure 18: Preliminary remote sensing analysis of heat wave impacts in Central Ontario, using S10 composite images of normalized difference vegetation index (NDVI) derived from SPOT VGT data (available online at http://free.vgt.vito.be/). Images show the differences between NDVI in 2010 and in an average 'standard year' (2004-2008 average) for two dates before and two dates after the late May heat wave. Where differences are positive (e.g. 2010 NDVI > 2004-2008 average NDVI) pixels are white; where differences are negative pixels are black. Black areas thus represent pixels which were 'less green' during 2010 than during an average year.