THE ROLE OF LOW TEMPERATURES IN DETERMINING THE NORTHERN RANGE LIMIT OF KUDZU (*Pueraria montana* var. *lobata*), AN INVASIVE VINE IN NORTH AMERICA

by

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
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Abstract

The role of low temperatures in determining the northern range limit of kudzu (Pueraria montana var. lobata), an invasive vine in North America

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Invasive non-indigenous species are among the principle drivers of global change, altering nutrient cycles, changing disturbance regimes, and generally threatening biodiversity. Climate change is widely expected to exacerbate invasions by relaxing abiotic barriers, such as low temperature, but the mechanistic evidence supporting this is limited. Here, I evaluate the hypothesis that low temperatures determine the northern range limit of kudzu (Pueraria montana var. lobata), an invasive Asiatic vine in North America, by assessing freezing and chilling tolerance of kudzu plants in winter, spring, summer, and fall. Kudzu was widely planted throughout the southeastern U.S. in the early 20th Century to prevent erosion. It is winter-deciduous and reproduces primarily from buds on stem nodes. In the last 40 years, kudzu has migrated northward in concert with a northward shift in the $-20 \degree C$ minimum winter temperature isocline, indicating that less severe winter cold is permitting northward migration. Freezing mortality during winter does not explain this correlation. Electrolyte leakage assays demonstrate that above- and belowground kudzu stems can survive to $-27 \degree C$ and $-17 \degree C$. Insulation provided by soil and snow protects belowground stems from lethal temperatures to well north of kudzu’s current range limit. Severe spring chill stops growth and photosynthesis and causes some shoot mortality, but both growth and photosynthesis recover quickly following the chill. Summer growth rates are rapid (up to 22 cm d$^{-1}$), responding within hours to tempera-
ture changes, and are unimpaired by nighttime lows. Photosynthesis is reduced at cool temperatures, but on cool days, kudzu leaves tend to be warmer than air temperature, so photosynthesis rates generally remain close to optimal values. In autumn, growth stops below 15°C, but leaves are retained and maintain modest photosynthetic competence until killed by frost in November. In colder climates that occur far north of kudzu’s current range, reductions in the length and quality of the growing season could accumulate over time to reduce kudzu’s success. There is, however, no strong evidence that low temperatures in any season will prevent kudzu from migrating throughout southern Ontario, making kudzu a good candidate for invasive species regulations.
To my parents.
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Chapter 1

Literature review

This chapter is the product of a collaboration with Rowan F. Sage, who contributed ideas and helped with the writing. Section 1.2 of this chapter will be modified for publication in collaboration with Cory Lindgren, Karen Castro, Robert Nurse, and Stephen Darbyshire. This section includes kudzu life history observations I made with the assistance of Albert Tenuta and Robert Nurse (contributions noted in the text) that do not appear elsewhere in this thesis.

Biotic invasions are one of the principle drivers of global change (Vitousek et al., 1997). Invasive non-indigenous species have far-reaching effects that include biodiversity loss (MEA, 2005) and compromised ecosystem services (Pejchar and Mooney, 2009). Some invasive species can alter nutrient cycles (Vitousek et al., 1997), fire cycles (Mack and D’Antonio, 1998), hydrology (Egoh et al., 2009), or the light environment (Walker and Vitousek, 1991). With species declines come lost genetic diversity and thereby lost adaptive potential to other climate change drivers (Barrett, 2000). In this way, invasive species undermine global ecological and economic capital needed for the biosphere to adapt to a changing climate.

Climate change is widely expected to exacerbate invasion frequency, extent, and in-
tensity (Dukes and Mooney, 1999; Mooney and Hobbs, 2000; Walther et al., 2001). Many invasive species have traits, such as strong responses to \( CO_2 \), that permit them to respond more rapidly to environmental changes than indigenous or non-invasive species (Dukes and Mooney, 1999). Other species, such as *Bromus tectorum*, interact with increased temperatures, rising \( CO_2 \), or other climate change drivers to initiate positive feedback loops that accelerate species loss (Mack and D’Antonio, 1998; Sala et al., 2000). Biological invasions are most frequent where humans are active (Mack et al., 2000; Leprieur et al., 2008), so the same economic development that is causing climate change will also continue to mix biota. In terms of extent, invasive species are not exempt from the “fingerprints” of climate change that have been observed since the rapid, post–1960 increase in global temperatures (Walther et al., 2001; Parmesan and Yohe, 2003; Walther et al., 2009; IPCC, 2007). Rapid range shifts and dramatic reorganizations of communities should intensify as climates continue to warm (Walther, 2003b; Peterson et al., 2008; Dukes and Mooney, 1999; Mooney and Hobbs, 2000; Walther et al., 2001, 2009).

Despite the well-documented problems with invasive species (Elton, 1958; Mooney and Hobbs, 2000; Vitousek et al., 1997; Sala et al., 2000; MEA, 2005), political barriers to managing invasive species still exist (Perrings et al., 2010). The close association between biological invasions and human traffic and trade is partly responsible, because any effort to stop the one will necessarily slow the other (Leprieur et al., 2008). Late action against invasion is usually more costly than early action, particularly if the costs of lost genetic diversity and ecosystem services due to the invasion are taken into account (Naylor, 2000). The economic costs of late action against invasion are large and difficult to quantify because lost genetic diversity and ecosystem services are challenging to valuate. However, we also know that effective prevention should keep those costs in check (Naylor, 2000). A major hurdle to implementing early preventative measures is having reliable predictions of invasive species range shifts in a changing climate.

Reliable predictions of range shifts require knowledge of factors limiting species dis-
tributions. Observers of vegetation patterns have been interested in why species occur where they do for over 200 years (e.g., von Humboldt and Bonpland, 1807). The modern fields of biogeography and physiological ecology have emerged out of this interest. Biogeography concerns itself primarily with the empirical or theoretical description of patterns at the regional to global scale, while physiological ecology concerns itself primarily with mechanistic descriptions of patterns at the organismal to population scale. The urgent need for predictions of how species will move in a changing climate gives both fields renewed relevance, and yet most of the efforts to generate predictions at the landscape scale have come from biogeography, and not from ecophysiology.

The most widely used method for predicting species movements is climate envelope modeling. This technique uses correlations between species presence and current climate to parameterize a statistical model, which is then used to project species movements into new areas, or into the future, using the output of global circulation models (Guisan and Zimmermann, 2000). The technique has been heavily criticized because actual distributions are the result of climate, but also historical and stochastic factors, dispersal limitations, and biotic interactions (Gaston, 2009; Colwell and Rangel, 2009; Pulliam, 2000). Also, metapopulation theory predicts that populations may exist in unfavourable areas (sink populations) if they are maintained by migration from more viable populations (source populations) (Pulliam, 2000). Moreover, paleoclimatology shows that past climates often have no modern analogue (Jackson and Overpeck, 2000). The same may also be true in the future, so species could be confronted with novel combinations of climate variables (IPCC, 2007; Jeschke and Strayer, 2008; Kearney and Porter, 2009). For these reasons, climate envelope modeling may not produce reliable predictions in future climates.

Weaknesses in climate envelope modeling have led to calls for the integration of mechanistic information from physiological ecology into empirical models (Helmuth et al., 2005; Chown and Gaston, 2008; Kearney and Porter, 2009). The result has been the introduc-
tion of new methods, including pseudo-mechanistic climate envelope models like CLIMEX and forest-gap models (Sutherst and Bourne, 2009; Bugmann, 2001), hybrid models like PHENOFIT (Morin et al., 2007), and fully mechanistic niche models (Kearney et al., 2008; Friend et al., 1993). All of these modeling approaches attempt to describe demographic or phenological processes such as growth or budbreak as a function of climate. For some well-studied species, such as the cane toad, the model can be so detailed that spatial information is not used (Kearney et al., 2008). This type of model is the exception rather than the rule because sufficient physiological data is lacking for most species (Kearney and Porter, 2009; Bugmann, 2001). Still, it is not clear that fully mechanistic models of this type are necessary because species distribution limits, while complicated, still carry valuable information that can help inform predictions of future movements.

In this thesis, I develop a method that uses biogeographic correlations to generate a set of mechanistic hypotheses to explain the northern range limit of the invasive species kudzu (*Pueraria montana* var. *lobata*). These hypotheses link temperature to life history traits via physiological responses. Kudzu is an appropriate study system because its northern range limit is correlated with climate and anecdotal evidence suggests that it is cold sensitive. As an invasive species, kudzu may represent a relatively simple ecological system where biotic interactions are minimized. Its dispersal has been historically facilitated by humans, so it is also more likely to be at equilibrium with climate. Specifically, I hypothesize that kudzu’s northern range limit is determined by low temperature. My approach to testing this hypothesis differs from mechanistic species distribution modeling because it is not a modeling method, it does not ignore species biogeography and it does not seek to describe all of the physiological relationships that determine species fitness (Kearney and Porter, 2009). It differs from most ecophysiological work in that it concerns itself not with the biochemical mechanisms underlying physiological patterns, but on how physiological patterns relate to life history and climatic patterns at the current range edge. This link has always been implicit in ecophysiology, but I seek to make it
Chapter 1. Literature Review

6

explicit in this thesis.

In the remainder of this chapter, I will explain how Hutchinson’s niche concept provides a theoretical basis for understanding how biogeography can inform experiments in physiological ecology. I will follow with a detailed review of the history and life history of kudzu, which will help with the interpretation of my results. I will then briefly review the physiological ecology of low temperature, the climatic variable I will focus on. I will close this chapter with an outline of my objectives for this thesis.

1.1 The niche concept

Most efforts to predict species distributions rely heavily on the niche concept as stated in the Hutchinsonian duality (Hutchinson, 1957; Guisan and Zimmermann, 2000; Kearney and Porter, 2009; Colwell and Rangel, 2009). This duality (or mapping) is a formal statement of the relationship between four-dimensional (three spatial dimensions plus time) geographic space, and Hutchinsonian “n-dimensional” niche space (Fig. 1.1) (Hutchinson, 1978; Colwell and Rangel, 2009). Just as geographic space has axes corresponding to spatial and temporal dimensions, niche space has axes corresponding to abiotic and biotic dimensions (Fig. 1.1). The fundamental niche as conceived of by Hutchinson is an abstract multidimensional “hyperspace” that delimits only the abiotic requirements for species success (Hutchinson, 1957) (i.e., the rectangle in niche space in Fig. 1.1). These variables, which include factors like temperature and substrate type, are the ultimate
determinants of where species can occur on a landscape. In this classic view, when biotic variables like competition are added in, the fundamental niche is reduced to the \textit{realized niche}, that is, the region in niche space where the species actually occurs \textit{(i.e., the black points in niche space in Fig. 1.1)}. The \textit{potential niche} is where the species’ fundamental niche and available climate overlap in niche space \textit{(i.e., the top half of the grey rectangle in Fig. 1.1)} \textcite{Jackson and Overpeck, 2000; Monahan, 2009}. Relative to the fundamental niche, available climate space continually changes, so the portion of the fundamental niche that is also the potential niche changes as the climate varies \textcite{Jackson and Overpeck, 2000}. Whether the realized niche fills the potential niche depends primarily on the species’ ability to disperse and colonize new populations \textcite{Jackson and Overpeck, 2000}. The fundamental niche can also change if species adapt to environmental pressures at the range edge \textcite{Jump and Penuelas, 2005; Colautti et al., 2010}. \textcite{Ball et al., 1991} Modern formulations of the niche concept recognize that for some species, obligate biotic interactions mean that the fundamental niche cannot be strictly abiotic \textcite{Godsoe, 2010}. Also, the realized niche can be larger than the fundamental niche if sink populations in suboptimal habitats are maintained by immigration \textit{(sink-source dynamics)} \textcite{Pulliam, 2000}, or if species presence is facilitated by other species. \textcite{Pulliam, 2000} These and other modifications of the original concept can be accommodated by Hutchinson’s duality by including biotic variables in niche space, or by mapping some of the populations onto areas outside of the fundamental niche \textcite{Jackson and Overpeck, 2000; Colwell and Rangel, 2009}. Such modern refinements to the niche concept bolster its role as a working model for understanding the relationship between what a species needs and its distribution.

It is the distinction between “distribution” and “niche” that makes the Hutchinsonian duality powerful for predicting the consequences of climate change and biotic exchange on species distributions. “Climate envelope” or “habitat” modeling, models the current \textit{realized} niche using the Hutchinsonian duality based on correlations between species occurrences and realized climate variables in geographic space \textcite{Kearney, 2006}. These
Figure 1.1 – An illustration of the Hutchinsonian duality. Species occurrences within a hypothetical distribution in geographic space in the left panel map onto niche space in the right panel. Two abiotic axes are shown, but there is no limit to the number of abiotic variables in niche space. The rectangle in niche space shows the entire fundamental niche of the species along these two dimensions, but only the portion above the dashed line is represented in geographic space. The unoccupied portion of this upper half is the potential niche, the part that is occupied (represented by black dots) is the realized niche. The lower half of the fundamental niche represents organismal variability that could permit the species to colonize other geographic areas should dispersal limitations be lifted, or could permit the species to survive in a novel future climate. In modern usage, the niche axes are generalized to include obligate biotic variables, blurring Hutchinson’s original distinction (Colwell and Rangel, 2009).
relationships are mapped onto niche space and are assumed to represent the fundamental niche (Kearney and Porter, 2009). This niche is then mapped back onto another geographic area to project invasion risk (Jeschke and Strayer, 2008), or into future climates to project species distribution shifts (Guisan and Zimmermann, 2000). In Fig. 1.1, habitat modeling would capture part of the upper half of the fundamental niche (the realized niche), but not the remainder, which represents the potential niche, or habitat that is present in geographic space but that is not occupied. The lower half of the fundamental niche, which is not currently realized in geographic space, is also missing from this type of model (Fig 1.1). This lower half represents variability that could mean the difference between extinction and persistence given a sudden shift in climate like the anthropogenically-forced one we are currently experiencing—even if it has no contemporary or past analogue. Accurate predictions of future species distributions, then, depend on our ability to characterize the fundamental niche (Kearney and Porter, 2009; Helmuth, 2009).

Full description of the fundamental niche would be onerous because the complete set of dimensions would include all of the main and interacting effects of abiotic and biotic variables necessary for growth, reproduction, and survival (Godsoe, 2010; Kearney and Porter, 2009). These factors often differ between life stages, making it impractical to generate detailed response surfaces covering all dimensions for every species we are interested in. However, this level of detail may not be necessary. Instead, prior knowledge, such as where species are, can be used to develop a targeted set of hypotheses that represent the most likely factors limiting organismal establishment, survival, and reproduction. If there is a strong correlation between a range limit and a climatic variable, then knowledge of the history and biology of the species in question can narrow the field of potential critical factors.
1.2 The history and biology of kudzu

Kudzu (*Pueraria montana* var. *lobata* (Willd.) Ohwi) is a perennial semi-woody vine native to east Asia (van der Maesen, 2002; Forseth and Innis, 2004). It was introduced to North America in 1876 at the Philadelphia Centennial Exposition, eventually rising from obscurity to become first a “saviour” and then a “scourge” in the southeastern U.S. (Blaustein, 2001; Winberry and Jones, 1973; Frankel, 1989; Shurtleff and Aoyagi, 1985). During its first 55 years in North America, kudzu shaded porches and fed livestock, but it was hardly popular; a mere 4,000 ha were planted (Winberry and Jones, 1973). Kudzu’s heydey came when the Soil Conservation Service, a branch of the U.S. Department of Agriculture, promoted kudzu as an ideal cover crop for southern soils denuded by decades of intensive cotton and tobacco farming (Bailey, 1939; Mitich, 2000; Winberry and Jones, 1973). Local radio celebrities agreed, recruiting 20,000 people to join the “Kudzu Club of America” (Alderman, 2004). At the peak of this campaign, the U.S. government paid landowners $8 acre$^{-1}$ ($20 ha$^{-1}$) to plant over 85 million kudzu seedlings, grown from imported and then, during World War II, domestic seed (Forseth and Innis, 2004; Winberry and Jones, 1973). Kudzu covered 1.2 million ha by 1946 (Blaustein, 2001). By the mid-1950s, enthusiasm waned as post-war wealth and new cover crops brought kudzu’s drawbacks into relief; young plants must be planted by hand and cultivated until they establish, and the tough vines make it difficult to bale (Winberry and Jones, 1973; Bailey, 1939). Most importantly, kudzu proved difficult to contain (Blaustein, 2001; Winberry and Jones, 1973). In 1970, twenty years after its popularity declined, kudzu was classified as a common weed; nearly thirty more years were required to declare it a noxious weed (Blaustein, 2001). Kudzu now covers up to 3 million ha in the eastern U.S. (Blaustein, 2001; Forseth and Innis, 2004).
1.2.1 Ecological and economic impacts

The main ecological effect of kudzu is the reduction of biodiversity by shading (Fig. 1.2). Like many woody vines, kudzu utilises the mechanical support of trees and man-made structures to minimize investment in its own supporting tissues, allowing it to allocate more biomass to vine elongation and leaf growth (Schnitzer, 2005; Forseth and Innis, 2004). Fast growth rates of up to 30 cm d$^{-1}$ or more allow kudzu vines to easily overtop even rapidly growing tree species (Forseth and Innis, 2004; Shurtleff and Aoyagi, 1985). Multiple layers of overlapping vines result in leaf area indices ranging from 3–4 m$^2$ m$^{-2}$ (in Nose, Japan, 34.82°N, 134.47°E) to 3.7–7.8 m$^2$ m$^{-2}$ (in Athens, Georgia, 33.95°N, 83.32°W) (Tsugawa et al., 1993b; Wechsler, 1977)$^2$. As a consequence, kudzu plants support 10–15 times more leaf area per unit stem biomass than mature deciduous trees (Forseth and Innis, 2004). The thick kudzu canopy (up to 2.5 m) results in the nearly complete interception of photosynthetically active radiation and the death by shading of underlying vegetation (Forseth and Innis, 2004).

In North America, kudzu’s growth habit makes it an economic liability and a public nuisance. Kudzu is responsible for up to $350 million annually in lost timber (Blaustein, 2001). Power companies spend an estimated $1.5 million per year removing the vines from power lines to prevent power disruptions from downed poles (Blaustein, 2001; McClain et al., 2006). Trains have been derailed by vines overgrowing train tracks (Blaustein, 2001; Shurtleff and Aoyagi, 1985). Landowners despise kudzu because it is difficult to keep off of buildings and out of crops, and because an established kudzu stand takes good pasture and agricultural land out of production (Blaustein, 2001; Shurtleff and Aoyagi, 1985).

Kudzu also produces both precursors for tropospheric ozone formation. Ozone is formed when isoprene, a small volatile hydrocarbon (C$_5$H$_8$) reacts with nitric oxide (NO)

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$^2$Tsugawa’s papers are available at CiNii, Japan’s scholarly and academic information navigator, http://ci.nii.ac.jp/vol_issue/nels/AN00194108_en.html
and other nitrous oxides in the light (Hickman et al., 2010). Kudzu leaves are prolific emitters of isoprene; emissions increase sharply with temperature, irradiance, and water stress (Sharkey and Loreto, 1993). Isoprene is a substantial sink for carbon, accounting for as much as 20% of fixed carbon at 35°C and 50% sunlight (Sharkey and Loreto, 1993). Kudzu is also the dominant nitrogen-fixing plant (over cultivated soybean) in the southeastern U.S., fixing up to 235 kg N ha⁻¹ (Hickman et al., 2010). Symbiotically fixed N leaks into infested soils and accelerates nitrification and denitrification, which are processes that emit NO and other nitrous oxides to the atmosphere (Hickman et al., 2010). Kudzu’s high N-fixation rate means that nitrous oxides are emitted from kudzu-invaded soils at a rate that is double that of uninvaded plots (Hickman et al., 2010). Kudzu’s unusual ability to produce both ozone precursors mean that kudzu invasions could lead directly to an increase in high ozone events, potentially undermining air quality legislation (Hickman et al., 2010).
Chapter 1. Literature Review

1.2.2 Species description and distribution

_Pueraria_ is a polyphyletic genus in the family Fabaceae (subfamily Faboideae) that should be divided into at least four genera (Lee and Hymowitz, 2001; Stefanović et al., 2009). Kudzu is located within the subtribe Glycininae in a strongly supported sister clade to soybean (_Glycine max_), an important annual crop species (Stefanović et al., 2009; Lee and Hymowitz, 2001). Up to 25 kudzu stems originate from rooted stem nodes called crowns (H.A. Coiner, personal observation), while buds at aerial stem nodes generate up to three branches (Tsugawa et al., 1988). Aboveground stems can overwinter to contribute to canopy grow-out in the spring, but the most important perennating organs are the crowns, which can be at or near the surface (under leaf litter), or a few centimetres underground (H.A. Coiner, personal observation). The canopy is supported by an extensive tuberous root system made up of roots that are up to 2 m long, 30 cm in diameter and weigh up to 180 kg (Shurtleff and Aoyagi, 1985; Miller and Edwards, 1983). It does not do well in areas with sandy, swampy, or shallow soils (Bailey, 1939). Young vines are herbaceous, green to purple, and covered with bronze hairs. Vines lignify and develop a smooth gray bark in the first growing season.

Kudzu leaves are large, alternate, and tri-foliate, with entire or broadly lobed (2–3) leaflets (McClain et al., 2006; Mitich, 2000). The inflorescence is a showy raceme-like panicle (10–20 cm long) with many reddish-purple flowers that smell like Concord grapes (Fig. 1.3B) (Mitich, 2000). Hymenoptera appear to be the primary pollinators (Thornton, 2004; Abramovitz, 1983). Flowers form dry, flat, hairy indehiscent pods up to 12 cm long containing few to several hard-coated kidney-shaped seeds. Fruits collected (by Albert Tenuta) in Leamington, Ontario in November 2009 were 1.5–11 cm long and contained 0–10 seeds. Samples of 25 seeds weighed an average of 0.30 ± 0.02 g. This is comparable to fruits collected in Illinois (McClain et al., 2006).

Kudzu is believed to have originated in China where it has been used for over 2000 y for fiber, food, and medicine (Shurtleff and Aoyagi, 1985). It was reportedly taken to
Figure 1.3 – Kudzu leaves (A) near Forrest City, Arkansas (2008), showing variation in lobing. Kudzu inflorescences (B) being visited by a hymenoptera in late July 2009 near South Haven, Michigan (H.A. Coiner).

Japan around 600 A.D., where it is prized primarily for culinary starch and fiber (Shurtleff and Aoyagi, 1985). Kudzu’s current native range extends from southeastern Asia north into the northeastern provinces of China and to Hokkaido (44°N), the northernmost Japanese island (van der Maesen, 2002; Shurtleff and Aoyagi, 1985). It now grows wild in the mountains of Japan up to 1000 m, and in lowlands and on small islands (Shurtleff and Aoyagi, 1985).

In North America, kudzu also has a wide distribution. Part of this is due to widespread plantings, but these were concentrated in the South where soils were poorest. Most of kudzu’s range expansion northward has happened after government-sanctioned planting ceased. Kudzu is still most prevalent where it was most widely planted—south of the Mason-Dixon line (dividing Maryland and Pennsylvania) especially in the heavy clay soils of Georgia, Alabama, and Mississippi (Sasek and Strain, 1990; Winberry and Jones, 1973). An experimental plot planted in 1924 in Ohio failed because of cold temperatures
(Williams, 1924), the same was apparently true in the 1950s for New York and Pennsylvania (see references in Winberry and Jones, 1973). A U.S. Department of Agriculture map from 1971 draws kudzu’s northern range limit running from southern Delaware west through southern Pennsylvania and the southernmost tips of Indiana and Illinois (U.S. Department of Agriculture, 1971). In 1981, populations were discovered in Massachusetts and Connecticut (Sorrie and Perkins, 1988); by 1989, kudzu was found in most New York City boroughs (Frankel, 1989). By the turn of the 21st century, there were over 78 kudzu populations in Illinois, including a few in the northern part of the state (McClain et al., 2006). Kudzu is now prevalent in Ohio (Hunt, 2011). A population in Leamington, Ontario was discovered in 2009, and is at least seven years old (see Section 1.2.8). The expansion of kudzu into areas where it was previously excluded by cold temperature occurred in a time period (1960–2000) that also saw climate warming (Wolfe et al., 2008). This correspondence strongly suggests that kudzu’s distribution is limited by low temperatures.

1.2.3 Cold tolerance

Kudzu is widely believed to not tolerate cold temperatures. Most authors describe kudzu as requiring a “mild” winter and a long hot growing season (Winberry and Jones, 1973; Miller and Edwards, 1983; Shurtleff and Aoyagi, 1985). Observations that leaves are killed by the first autumn frost are taken to mean that kudzu has a “high sensitivity” to winter cold (Carter and Teramura, 1988a). The apparent die-back of aboveground vegetation is also interpreted as indicating frost sensitivity (Mitich, 2000; Sorrie and Perkins, 1988). Claims of high sensitivity to cold are inconsistent with the additional observation that kudzu flourishes in many cold places. In both North America and Japan, kudzu’s northern range limit corresponds to the -20°C minimum winter temperature isocline (Sasek and Strain, 1990). In Korea, kudzu reportedly grows where temperatures drop to -30°C; roots are said to survive to -25°C (Shurtleff and Aoyagi, 1985). This
inconsistency has not stopped authors from hypothesizing that kudzu's northern spread is limited by winter temperatures (Sasek and Strain, 1990; Forseth and Innis, 2004; McClain et al., 2002). Evaluating this hypothesis is currently impossible because of a lack of physiological data.

1.2.4 Canopy development and shade avoidance

In North America, bud burst of kudzu occurs from late March (in Valdosta, Georgia, 30.78°N, 83.28°W) to mid-May (in Leamington, Ontario, 42.02°N, 82.74°W) (H.A. Coiner, personal observation). Once stem emergence begins, the canopy matures rapidly. In Kitabata, Japan (36.60°N, 130.72°E), stems emerged on April 3, 1982 (Tsugawa et al., 1987). Twenty-three days later, 75% of the current year’s stems had been produced. No stems were initiated past the beginning of June (Tsugawa et al., 1987). This is consistent with another study that showed that kudzu takes about two months to achieve full canopy maturity from first leaf-out, in terms of dry weight, total stem length, leaf area index and specific leaf area (Tsugawa et al., 1993b). If this holds in the northern part of its North American range, then the kudzu canopy should reach its peak in mid- to late July.

Kudzu’s pattern of canopy development maximizes light capture. A single crown can produce stems with a range of morphologies: some stems are thin and pale with closely-spaced nodes while others are thick and succulent with widely-spaced nodes. All stem nodes have three buds, but whether they initiate branches depends on the buds’ location relative to each other, their location relative to the stem tip, and the age of the originating stem. Branching is rare in current year’s stems. At an experimental plot in Kobe, Japan (34.68°N, 135.20°E), most (85%) of the buds on the current year’s stems remained dormant (Tsugawa et al., 1988). The following year, about 40–50% of the dormant nodes generated at least one branch, most of the remainder were still dormant (Tsugawa et al., 1988). The lateral buds are more likely to branch in the second year after the middle bud bursts or aborts (Tsugawa et al., 1988). Stems older than two years
are rarer, so they don’t contribute as much to the current year canopy (see Section 1.2.8, Tsugawa et al., 1987). In terms of dry weight, most (76%) of the current year’s stems emerge from stems initiated the previous year (Tsugawa et al., 1987). Buds are also more likely to branch if they are on the middle or upper parts of the stems. Second year buds on the upper portions of stems branched 25–36% of the time, while buds on the lower portions of stems branched 7–25% of the time. Middle portions produced 17–47% of current year’s stems (Tsugawa et al., 1988). The result of these patterns is that stems elongate in the first season and branch in the second from buds on the middle to upper portions of the stem. This branching pattern maximizes foliage production in the upper reaches of the canopy where light is available. At the same time, it maintains dormant buds in the lower canopy that are then capable of regenerating a canopy if the stem is damaged (Tsugawa et al., 1988).

Kudzu does best in full sunlight. Compared to two other exotic vine species and five native vines grown in low light in a forest understory, kudzu had the highest light compensation point of photosynthesis (43 ± 10 µmol−1 m−2 s−1), which is the amount of light it needs to offset carbon lost to respiration (Carter and Teramura, 1988b). Seedlings grown in 13% sunlight have fewer branches that take 50–100% longer to emerge than control seedlings grown in unshaded glasshouse conditions (79% sunlight) (Tsugawa et al., 1985). Shaded seedlings also had 22% lower shoot dryweight, fewer branches, and about 30% of the total stem length of control plants (Tsugawa et al., 1985). At intermediate levels of shade, the main shoot tended to be longer (but flimsier) than either the controls or the most intense level of shade (Tsugawa et al., 1985), which suggests that moderately-shaded kudzu stems can increase their growth rate to seek sun. Leaves emerge more slowly in deep shade, with total leaf area dropping to 37% of the controls, and leaflet dry weight decreasing to about 20% of the controls (Tsugawa et al., 1985). Root basal diameter of deeply shaded plants was about half that of control plants, while total root dry weight was 15% that of the controls (Tsugawa et al., 1985). Similar results were found in the field.
Abramovitz (1983) measured seasonal growth characteristics at three sites varying in sun exposure in Maryland. The west-facing site, which received at least 75% full sunlight (> 1500 µmol m⁻² s⁻¹) from June through August, had greater aboveground biomass, greater specific leaf weight, and greater leaf area index than the north-facing site, which became shaded in August, or the east-facing site, which never received more than ca. 700 µmol m⁻² s⁻¹, or 33% full sunlight Abramovitz (1983). These observations show that kudzu performance depends on access to full sunlight.

1.2.5 Leaf angles and drought avoidance

Kudzu leaves change their leaf angle rapidly with respect to the sun. Steep leaf angles (60°–80° from horizontal) are thought to help the kudzu canopy maximize photosynthesis while minimizing damage due to high temperatures, water loss, and excess light (Forseth and Innis, 2004; Forseth and Teramura, 1986). At midday, leaves orient away from the sun, lowering leaf temperatures by 5–6°C relative to air temperature (Forseth and Teramura, 1987). When artificially restrained, higher leaf temperatures are associated with 30% increased stomatal conductance (Forseth and Teramura, 1986). Higher conductance leads to higher transpiration rates, which is an alternative way of cooling the leaf, as long as water is readily available (Forseth and Teramura, 1986, 1987). Steep leaf angles also occur at moderate temperatures, however, which would be expected to reduce photosynthetic capacity because steep angles would reduce absorbed light intensity (Forseth and Teramura, 1986). This might be offset by another effect of steep leaf angles, which is to allow unintercepted light to penetrate to lower leaf layers, allowing lower canopy leaves to maintain maximum rates of photosynthesis (Forseth and Teramura, 1986). Photosynthesis in sun-acclimated leaves saturates at about 50% of full sunlight (1000 µmol m⁻² s⁻¹) (Forseth and Teramura, 1987), hence steeply angled leaves at the top of the canopy could still operate near light saturation while allowing photons to penetrate to lower canopy layers. The net benefit is a high canopy-level carbon gain.
Despite steep leaf angles, midday wilting is nevertheless common in kudzu populations throughout the northeastern and midwestern United States (H.A. Coiner, personal observation). This could indicate that kudzu shoots experience severe water stress; however it likely reflects an adaptation to reduce xylem cavitation. Kudzu petiole resistance is 20–100 times greater than internode resistance (Taneda and Tateno, 2007), which would restrict water delivery to heavily transpiring leaves (causing wilting) while at the same time preventing cavitation in the xylem (Tyree and Sperry, 1989). Hydraulic conductance in kudzu is also facilitated by substantial transverse movement of water in xylem (Taneda and Tateno, 2007), which could act as a safety mechanism to get around damaged xylem (Tyree et al., 1994). Diurnally reversible kudzu leaf wilting may also allow avoidance of high leaf temperatures rather than water stress because wilted leaves intercept less light, reducing the solar heat load on leaves.

Kudzu has a limited ability to avoid drought stress. Depression-era farmers without irrigation systems liked kudzu because it could withstand episodic dry spells (Bailey, 1939). Compared to five other native and exotic vines, kudzu has the strongest response to dry air; photosynthesis was reduced to 40% of its maximum at a leaf-to-air vapor pressure difference of 4 kPa (Carter et al., 1989). Kudzu probably avoids short dry periods by strictly controlling its stomates, and by maintaining a deep and succulent root system (Wechsler, 1977; Forseth and Teramura, 1987). In particular, the capacitance of kudzu’s large roots could help buffer against fluctuations in water status (Goldstein et al., 1984). If kudzu is similar to tropical lianas, it may also be able to maintain high photosynthesis rates during dry spells (Cai et al., 2009). Still, these drought avoidance mechanisms are not sufficient to permit kudzu colonization of arid areas. In Japan and in North America, kudzu’s habitat is associated with annual precipitation of at least 100 cm (Sasek and Strain, 1990; Shurtleff and Aoyagi, 1985), and aridity is hypothesized to prevent the westward range expansion of kudzu (Sasek and Strain, 1990). This biogeography is consistent with that of most other temperate vines in North America, which are thought
to be confined to the wet climates of the southeastern U.S. because vine xylem is more vulnerable to cavitation relative to other woody species (Teramura et al., 1991; Tyree and Sperry, 1989). Kudzu’s vulnerability to drought-induced cavitation has not been studied, but its xylem vessels are large (up to 300 µm in diameter), suggesting that they might be susceptible to cavitation (Tyree and Sperry, 1989; Wechsler, 1977). Leaf anatomy is also not particularly xerophytic. Leaves have a trichome density of 8 mm\(^{-2}\), a cuticle thickness of about 1 µm, and about 5 times more stomates on the lower versus the upper epidermis (Pereira-Netto et al., 1997). Stomatal control is tight, but that itself is not sufficient to help kudzu tolerate areas with low rainfall because closed stomates also prevent the influx of CO\(_2\).

In addition to preventing CO\(_2\) uptake through stomatal closure, drought also increases carbon lost to isoprene emission. Drought-stressed kudzu leaves emit up to 5 times more isoprene than unstressed leaves (Sharkey and Loreto, 1993). Unlike photosynthesis, which recovers to control levels within five days following drought stress, isoprene production remains high for about 12 days (Sharkey and Loreto, 1993). This amounts to the loss of about 14% of total carbon uptake (Sharkey and Loreto, 1993).

### 1.2.6 Photosynthesis response to temperature

Published rates of maximum net CO\(_2\) assimilation (\(A_{\text{net}}\)) for kudzu are typically between 11 and 27 µmol\(^{-1}\) m\(^{-2}\) s\(^{-1}\) (Wechsler, 1977; Forseth and Teramura, 1987; Sharkey and Loreto, 1993; Carter et al., 1989). This is similar to soybean but at the low end of the scale for other fast-growing C\(_3\) weeds and crops such as Chenopodium album and sunflower (Bunce, 2000). Kudzu may be able to compensate for its moderate \(A_{\text{net}}\) by permitting light to penetrate to lower leaf layers (see Section 1.2.5). The thermal optimum for photosynthesis is broad, between 25° and 30°C (Wechsler, 1977; Forseth and Teramura, 1987; Sharkey and Loreto, 1993). \(A_{\text{net}}\) does not drop much at 20°C. In plants grown at 19–20°C in Wisconsin or at \(\sim\) 25°C in Maryland, net photosynthesis at 20°C
was depressed by about 5–10% relative to $A_{\text{net}}$ at the thermal optimum (Forseth and Teramura, 1987; Sharkey and Loreto, 1993). At temperatures < 20°C, the temperature response of warm-grown kudzu shows some evidence of decline; in plants grown at $\sim 26^\circ$C in Georgia, $A_{\text{net}}$ dropped by 20% at 18°C relative to the temperature optimum (Wechsler, 1977). No study has measured the temperature response of photosynthesis at temperatures below 18°C.

Net CO$_2$ assimilation is also sensitive to high temperatures. Photosynthesis in plants grown at $\sim 20^\circ$C drops to 2/3 the maximum rate at 35°C, and falls to just 1/3 the maximum at 40°C (Sharkey and Loreto, 1993). This drop is more pronounced than that seen in plants grown in warmer conditions ($\sim 25^\circ$C) in Maryland. There, assimilation dropped by 10–15% when leaves reached 35°C, and by 50% at 42°C, compared to assimilation at the temperature optimum (26°C) (Forseth and Teramura, 1987). However, assimilation in field-grown plants in Georgia (at $\sim 26^\circ$C) was about 60% of the optimum at just 36°C (Wechsler, 1977), a much larger drop than that seen in plants grown in similar conditions in Maryland. C$_3$ plants commonly show depressed photosynthesis rates above 35°C, but warm-climate species like tobacco and rice show modest declines (5–18%) at 35–40°C (Makino and Sage, 2007; Sage and Kubien, 2007). This high temperature sensitivity may seem inconsistent with kudzu’s success in the southern U.S. where summer air temperatures frequently exceed 35°C. The ability to regulate leaf temperature with leaf angle (Section 1.2.5) may account for this discrepancy.

1.2.7 Sexual reproduction

Sexual reproduction does not appear to be an important part of kudzu life history, but kudzu produces viable seed in many northern populations, so seeds could be important dispersal agents. Flowers develop in mid- to late summer in both northern and southern populations; fruit sets in early fall. The Leamington, Ontario population flowered every year between 2009–2011 (H.A. Coiner, personal observation). All 78 populations in
Illinois described by McClain (2006) flowered in the summer of 1997. Eight of the 14 northernmost kudzu populations visited in August 2009 were already in flower (H.A. Coiner, personal observation). Flowering may be related to sun exposure or to patch size. Of three kudzu sites studied in Maryland, the two that produced the most inflorescences (up to 15.2 inflorescences m$^{-2}$) also had higher incident radiation and were much larger than the third, which produced only two inflorescences in total (Abramovitz, 1983). Some authors report flowers on climbing stems only (e.g., Britton et al., 2002), but Abramovitz (1983) observed inflorescences on both climbing and trailing stems. Both types of vines also flower in Leamington, Ontario (H.A. Coiner, personal observation).

Not all flowers set seed. In Illinois in summer 1997, only six of the 78 populations that flowered produced mature fruits (McClain et al., 2006). In Leamington, Ontario, nearly 17% of seeds were mature (out of 1952 seeds in 506 pods, collected by Albert Tenuta). More seeds matured in pods initiated near Lake Erie (30%) than upslope near a cultivated field (8%) (Fig. 1.2B, H.A. Coiner, personal observation), providing some evidence to support the hypothesis that microclimate can influence fruit maturation (Pappert et al., 2000). Seed set requires pollinators: flowers that were excluded from pollinators produced no pods, but the maximum seed set for naturally-pollinated kudzu flowers in Maryland was only 3.3% (Abramovitz, 1983). This increased to 14% with hand-pollination and pod set increased to 20–40%, suggesting pollinator limitation (Abramovitz, 1983). Still, the increases are modest and not due to poor pollen, which was > 95% viable, so pollen limitation is not the whole story. Seed set may depend instead on within-stand genetic diversity, or the potential for outcrossing (Pappert et al., 2000). Preliminary results of allozyme work done by Jim Hamrick suggest that the Leamington, Ontario population is more genetically diverse (with 85% polymorphic loci) than any of the other 30 North American populations collected (J. Hamrick, personal communication). The association of high genetic diversity with (relatively) high seed set at Leamington is consistent with the hypothesis that seed set is outcrossing dependent.
Kudzu seeds are viable but seedlings are rare. Germination in unscarified seed ranges from 11–32% (Susko et al., 2001; McClain et al., 2006). Once scarified, germination rates are high, at 94–100% for Illinois seeds, 100% for Leamington seeds, and 80–93% for North Carolina seeds (R. Nurse, personal communication, Susko et al., 2001; McClain et al., 2006). Seedlings are thought to be delicate (Forseth and Innis, 2004) but planted scarified seeds develop into vigorous seedlings (McClain et al., 2006). However, seedlings in nature are rare, both in North America (Forseth and Innis, 2004) and in Japan (Tsugawa and Kayama, 1985). No seedlings have been found near the kudzu site in Leamington, Ontario (R. Nurse, personal communication) despite plenty of viable seed. When seedlings are found, none survive to the following year (Abramovitz, 1983). The absence of seedlings implies that seeds are not dispersed effectively, or that there is some other barrier to seedling establishment. Pods can disperse up to 25 m, but most (92%) stay within 6 m of their origin (Abramovitz, 1983), and there is no obvious animal vector, although mammals browse kudzu and could ingest seeds (H.A. Coiner, personal observation). Overall investment in sexual reproduction is also low, at just 0.02–2.1% of summer carbon gain in southern populations (Wechsler, 1977; Forseth and Innis, 2004). Kudzu in Japan is no different; flower clusters and fruits make up only 0.7% of total aboveground dry weight (Tsugawa et al., 1993b). This low investment, combined with low seed set and low recruitment suggests that sexual reproduction and subsequent seedling recruitment are not major players in kudzu’s life history.

1.2.8 Asexual reproduction and population structure

Vegetative reproduction via rooted stem nodes is widely thought to be the principle reproductive mode (Tsugawa and Kayama, 1985; Forseth and Innis, 2004). About 25–45% of stem nodes take root, with rooting being more common in the middle portion of the current year’s stem than in the upper or lower portions (Tsugawa et al., 1988). The combination of extensive branching and rapid degradation of connections between nodes
on a single stem can lead to densities of tens of thousands of kudzu plants per hectare (Mitich, 2000). Crown densities of 6.3–20.7 crowns m$^{-2}$ have been observed in Japan, which amounts to as many as $2 \times 10^6$ crowns ha$^{-1}$ (Tsugawa et al., 1992). Many of these crowns may be independent individuals. Tsugawa and colleagues (1985) define an individual plant as a unit that is capable of independent growth, which in kudzu occurs if the stem connecting it to the parent plant dies or becomes severed. The distribution of plant length and weight in one Japanese patch is vast, ranging from 0.01–62.7 m and 0.5–4231 g (Tsugawa and Kayama, 1985). That said, most of the plants are small: about three-quarters of overwintering stems in natural Japanese kudzu populations had three or fewer vascular rings, 53% were less than 1 m in length and about 60% weighed less than 100 g (Tsugawa and Kayama, 1985). This suggests that detachment of plants from parents occurs frequently, especially when crowded (Tsugawa and Kayama, 1985). Many more plants develop when parent plants are closely (40 cm) rather than widely (120 cm) spaced (Tsugawa et al., 1990). Widely spaced plants quickly cover the available space, but don’t necessarily sever physiologically from the rooted nodes until crowding occurs.

In the clonal herb *Trifolium repens*, plants share patchy resources through their stem connections (Stuefer et al., 1996). If kudzu does this as well, maintaining connectivity could aid plant establishment.

This population structure makes age determination a challenge. The largest of roots exposed by a heavy storm in Leamington had seven vascular rings at its base (H.A. Coiner, personal observation), but ring number may be an unreliable indicator of population age. At a 15-y-old stand in Nose, Japan, the largest stem found in extensive sampling had just five rings (Tsugawa et al., 1993a). There may even be a maximum ring number for kudzu plants. At four natural stands in Japan, all stems and roots in a $10 \times 10$ m$^2$ area were sampled. The largest root at these sites had six rings and the largest stem had seven, while the majority of stems had just one ring (46–73%) (Tsugawa et al., 1992). The majority (59–86%) of overwintering stems at another natural patch
in Japan had just one vascular ring, while the largest stems (with five rings) were very rare (0–0.3%) (Tsugawa et al., 1987). Tsugawa et al. (1992) interpret these numbers to indicate the maximum age of kudzu stems and roots, but not the population. They speculate that the high proportion of one- and two-ring stems and roots in these stands indicates high turnover, and the ability to recover quickly from disturbance (Tsugawa et al., 1992), which is consistent with the much larger number of small than large plants in kudzu stands. The 2 m long, 200 kg roots of kudzu lore may be exceptions rather than the rule (Shurtleff and Aoyagi, 1985; Miller and Edwards, 1983).

1.3 The physiology of low temperature

In Section 1.2.2 I note that kudzu’s distribution may be limited by low temperatures. In this section, I will briefly review the low temperature physiology of plants. I divide low temperatures into three categories: freezing (< 0°C), chilling (0–12°C), and suboptimal (10–35°C).

1.3.1 Freezing temperatures

Freezing temperatures are commonly defined as temperatures < 0°C, but in nature, the temperature at which water freezes is highly variable. Rarely does water in the protoplast freeze at zero because native concentrations of solutes depress the freezing point to between −2 and −4°C (Levitt, 1980). Ice formation in plants requires external nucleation above the spontaneous nucleation temperature of −40°C (Pearce, 2001). Nucleators include debris, ice crystals, snow, ice-nucleating bacteria, and some biological molecules and structures (Pearce, 2001). In herbaceous plants, ice often nucleates on the plant surface and enters the leaf through stomata (Wisniewski et al., 2002; Pearce, 2001); in woody plants, intrinsic nucleators seem to be present, but are not yet characterized
(Wisniewski et al., 2008). Plants can either avoid nucleation by supercooling, or they can control nucleation and crystal growth, permitting ice to form safely in extracellular spaces (Levitt, 1980; Sakai and Larcher, 1987; Pearce, 2001). Woody plants that successfully overwinter tend to employ a combination of these strategies (Wisniewski et al., 2008).

Supercooling occurs when the freezing point of intracellular solutions is lowered by the accumulation of solutes, including di- and trisaccharides (e.g., sucrose and raffinose), amino acids (e.g., proline), soluble proteins, and sugar alcohols (e.g., sorbitol) (Kasuga et al., 2008; Levitt, 1980). Of these, soluble sugars may be the most effective at preventing freezing in acclimated tissues (Levitt, 1980). For example, cold-acclimated canola leaves contained up to twenty times the sugar content of unacclimated leaves, lowering their freezing temperature from $-3$ to $-17\,^\circ C$ (Gusta et al., 2004). In woody plants, sugar concentrations can be proportional to freezing tolerance, with buds often being the most protected (Levitt, 1980; Sakai and Larcher, 1987). Nucleation must also be prevented in supercooling tissues. Plants do this by removing nucleators from the protoplast, or by increasing concentrations of anti-nucleating agents like flavonol glycosides (Guy, 1990; Kasuga et al., 2008). When the supercooling capacity is exceeded, ice forms intracellularly, killing the cell. Some deep supercooling tissues, like apple xylem parenchyma, can avoid freezing down to $-40\,^\circ C$, when the water freezes in the absence of a nucleator (Ashworth et al., 1988; Pearce, 2001).

Plant tissues that tolerate extracellular ice formation can survive much colder temperatures than $-40\,^\circ C$. Some boreal species, like Cornus sericea, a dogwood, can survive to the temperature of liquid helium ($-269\,^\circ C$, Guy et al., 1986). Freeze-tolerant plants typically nucleate ice at warm subzero temperatures ($-0.5$ to $-2\,^\circ C$) in intercellular spaces (Pearce, 2001). Cold-acclimated plants are able to control both the speed and location of ice formation (Pearce, 2001; Gusta et al., 2004; Wisniewski et al., 2008). So-called “antifreeze” proteins may help direct this process (Griffith et al., 1992, 2003).
imaging shows that the initiation of ice formation, the propagation of ice through tissues, and the speed of ice propagation are all highly variable, depending heavily on physical properties of the tissue and its acclimation state (Ball et al., 2002; Wisniewski et al., 1997). In general, the slow, controlled growth of ice crystals is not directly damaging. Rather, the main cause of cell death in tissues that freeze is dehydration (Levitt, 1980). This occurs when the safely-formed ice draws water out of the cell down a vapor pressure gradient that increases as the ice cools (Ashworth et al., 1993; Pearce, 2001). If the protoplast dehydrates to a point where the plasma membrane structure is damaged, compartmentation is lost, which is detectable as leakage of electrolytes (Pearce, 2001; Steponkus, 1984). The point where this occurs defines the “freezing” (actually desiccation) tolerance of the tissue. Freeze-sensitive crop plants, like tomato and bean, also form ice in intercellular spaces, but the tissues cannot tolerate the associated dehydration (Ashworth and Pearce, 2002).

Supercooling and extracellular freezing are usually thought of as mutually exclusive traits (Karlson et al., 2004)—Arabidopsis supercools while winter rye freezes (Reyes-Diaz et al., 2006; Griffith et al., 1992). They even show evolutionary divergence; in dogwoods, supercooling is basal to extracellular freezing (Karlson et al., 2004). The evolution of extracellular freezing may have permitted dogwood invasions north of the $-40^\circ C$ winter isotherm, which forms the northern boundary of many of the supercooling species (Karlson et al., 2004). But there is also evidence that many woody species employ both strategies. Thermal imaging in cranberry (Workmaster et al., 1999), peach (Quamme et al., 1995) and blackcurrant (Carter et al., 2001) has revealed physical barriers restricting extracellular ice formation to tissues that can tolerate it, protecting supercooling tissues (often buds or flowers). Magnetic resonance imaging in cold hardy conifers (Ide et al., 1998) and a Rhododendron species (Price et al., 1997) shows the slow dehydration of buds during cooling, consistent with the migration of water out of the organ into neighbouring (frozen) tissue (Ishikawa et al., 1997). The plasma membrane is believed to
mediate whether equilibrium is achieved by continued dehydration or by intracellular ice formation, so a failure to survive freezing temperatures will be associated with membrane failure (Steponkus, 1984; Xin and Browse, 2000).

### 1.3.2 Chilling temperatures

Chilling temperatures are low, non-freezing temperatures (0–10°C) that can restrict carbon assimilation and growth in chilling-sensitive plants (Levitt, 1980). In nature, these temperatures are most likely to occur as short-term departures from an otherwise favourable temperature regime (Allen and Ort, 2001). Temperatures generally reach their minimum just before dawn, but on cold mornings, leaves are often still chilled when they first encounter the sun. The physiological effects of chilling in the dark differ from those in the light because chilling temperatures interact with light stress to inflict greater damage than chilling temperatures alone (Powles et al., 1983).

The main effects of chilling at night are stomatal closure, reduced Rubisco activity, and reduced activity of enzymes involved in carbohydrate metabolism (Allen et al., 2000; van Heerden et al., 2003; Bertamini et al., 2005). Chilling at night can often carry over to reduce plant performance during the day despite ameliorated temperatures. For example, overnight exposure of mango to 7°C reduced $A_{\text{net}}$ at warm midday temperatures by 50% compared to controls (Allen et al., 2000). Night chilling can also impact growth rates. For example, three nights of overnight chilling to 10°C reduced the relative growth rate of cotton seedlings by 25% during 3 days of recovery (DeRidder and Crafts-Brandner, 2008). These effects were reversible, but evidence from chilled grapevine buds indicate that rates of leaf emergence and shoot elongation may be reduced over the entire growing season, even if temperatures ameliorate (Keller, 2010).

The most damaging effects of chilling temperatures occur when chilling temperatures are combined with high irradiance (Powles et al., 1983; Allen and Ort, 2001). Light is both a blessing and a curse for plants (Demmig-Adams and Adams, 2000). Blessing,
because it provides the energy for photosynthesis; curse, because it can cause irreversible damage if the energy is not safely dissipated (Powles et al., 1983; Allen and Ort, 2001). Plants must therefore maintain a dynamic energetic balance between functional and damaging processes. Chilling temperatures act to disrupt that balance (Huner et al., 1998; Ensminger et al., 2006; Takahashi and Murata, 2008). If the balance is not righted through photoprotective mechanisms, or biochemical shifts (acclimation), then the excess light results in chlorophyll bleaching and, in extreme cases, leaf death (Powles, 1984).

The main target of light damage is photosystem II (PSII), the first step in the conversion of light energy to chemical energy (Huner et al., 1998; Long et al., 1994). Excess light inactivates the oxygen-evolving complex associated with PSII. Without that electron sink, electrons escaping from over-reduced chlorophylls attack the D1 protein at the core of PSII. This occurs even under favorable conditions, but in that case, D1 repair is efficient (Long et al., 1994). Chilling acts to disrupt repair by slowing enzymatic reactions, and by slowing the transcription and translation rates of new protein in chilling sensitive species (Allen and Ort, 2001). For example, chilling in tomato prevents the light-activation of carbon reduction enzymes like fructose-1,6-bisphosphatase that are critical in the regeneration of ribulose-1,5-bisphosphate (RuBP), a key substrate of Rubisco, and in the processing of starch and sucrose (Sassenrath et al., 1990; Taiz and Zeiger, 2006, see also Section 1.3.3). In other species, such as maize, chilling results in the gradual loss of protein relative to chilling-resistant species, such as Miscanthus (Naidu et al., 2003). Processes that depend on fast protein turnover, such as the conversion of light energy to chemical energy, which depends on D1, are particularly susceptible to slowed protein metabolism. This effect is exacerbated if Calvin cycle reactions are also slowed, sequestering NADP⁺, a key electron acceptor, and electrons get passed to oxygen instead. This creates peroxides and other reactive oxygen species that further inhibit the synthesis of D1 and thereby PSII repair (Takahashi and Murata, 2008; Murata et al., 2007). Chilling-resistant plants are able to protect PSII, either by scavenging the
reactive oxygen species that prevent PSII repair, or by dissipating excess energy as heat (Takahashi and Badger, 2011).

Absorbed light energy is dissipated either by photochemical processes ($\Phi_P$), or by a range of non-photochemical processes ($\Phi_{NP}$) (Kornyeyev and Holaday, 2008). The symbol $\Phi$ represents the "quantum yield" of a process, which, in this context, is the proportion of total absorbed light energy that is being directed to a particular process, so

$$\Phi_P + \Phi_{NP} = 1$$

Photochemical processes result in carbon fixation, while non-photochemical processes safely dissipate light energy as heat (Maxwell and Johnson, 2000). Changes in the relative magnitude of these processes can be studied using chlorophyll fluorescence techniques. Chlorophyll $a$, the pigment that donates electrons directly to PSII, re-emits about 1–2% of absorbed light energy as fluorescence (Taiz and Zeiger, 2006). This signal, which is itself a non-photochemical process, can be used to infer the fate of the rest of the light energy (Maxwell and Johnson, 2000; Ball et al., 1995; Kornyeyev and Holaday, 2008).

The most productive fate for light energy is photochemistry. The maximum quantum yield for photochemistry is measured using a fluorescence parameter called $F_v/F_m$. This parameter is measured in the dark just before dawn when the pool of $Q_A$, a protein that accepts electrons from PSII, is fully oxidized and all PSII reaction centres are presumably repaired and activated. This makes $F_v/F_m$ a sensitive indicator of damage sustained to the reaction centre (Maxwell and Johnson, 2000; Krause and Weis, 1984). The quantum yield for photochemistry in the light ($\Phi_P$) is always lower than $F_v/F_m$, but the difference can reflect protective as well as damaging processes. Even healthy plants must contend with the damaging effects of light, so daily reversible declines in $\Phi_P$, because $e.g.$, some PSII may have damaged reaction centres, are common (Long et al., 1994). A chilling-sensitive plant should not have many dynamic, rapidly inducible mechanisms for photoprotection, so declines in $\Phi_P$ should be sustained (Allen and Ort, 2001). This is because photodamage takes longer to repair, and in some cases, as with pigment bleach-
ing, the damage is permanent (Allen and Ort, 2001; Powles, 1984). $F_v/F_m$ should also decline if repair is inhibited (Maxwell and Johnson, 2000). Such sustained loss of the ability to capture light results in lost photosynthetic opportunity and, ultimately, slower growth rates (Long et al., 1994; Raven, 2011).

The quantum yield of non-photochemical processes ($\Phi_{NP}$) is divided into three components based on their kinetics (Kornyeyev and Holaday, 2008; Walters and Horton, 1991; Müller et al., 2001). Dynamic processes (relaxing in seconds to tens of minutes), including activation of the xanthophyll cycle, are represented by $\Phi_{Reg}$ (Demmig-Adams and Adams, 1996; Horton et al., 1996). The xanthophyll cycle uses excess light energy to convert the pigment violaxanthin into zeaxanthin; heat is released when the process reverses (Demmig-Adams and Adams, 1996). Processes that take tens of minutes to hours to reverse are represented by $\Phi_{NF}$. This parameter is generally associated with PSII deactivation, which could result from damage to and failure to repair D1 (Grennan and Ort, 2007), or, as in chilling-resistant evergreens, the protective dissociation of light harvesting complexes from PSII (Öquist and Huner, 2003; Horton et al., 2005). The third parameter, $\Phi_{f,CON}$, is the quantum yield of processes that take longer than one day to reverse. This is a grab-bag of chlorophyll $a$ fluorescence, and all of the constitutive (i.e., very slowly changing) dissipation mechanisms, including protective structural changes, and permanent photodamage (Gilmore and Ball, 2000; Kornyeyev and Holaday, 2008). These three parameters together represent the quantum yield of all of the non-photochemically-dissipated energy, such that

$$\Phi_{NP} = \Phi_{Reg} + \Phi_{NF} + \Phi_{f,CON}$$

Despite their mechanistic ambiguity, these fluorescence parameters are useful indicators of how flexible a species is in dealing with high light. For example, if non-photochemical dissipation is fast-relaxing (i.e., $\Phi_{Reg}$ is large) during a chilling treatment, the leaf would be using dynamic mechanisms to protect its photosynthetic apparatus, and has probably sustained little if any damage to the photosynthetic apparatus (Hendrickson et al., 2003).
1.3.3 Suboptimal temperatures

Suboptimal temperatures occur below a species-specific optimum, typically between 10–35 °C, but above values where chilling injury occurs (Berry and Bjorkman, 1980). They are usually non-damaging and the effects are often reversible once temperatures ameliorate (Sage and Kubien, 2007). The main effect of suboptimal temperatures is to slow down reaction times. For example, Rubisco, the primary carboxylating enzyme in plants, has a $Q_{10}$ near 2.2, which means that its catalytic capacity more than doubles with every 10 °C increase in temperature (Sage, 2002). Since every enzyme has its own temperature response, changes in temperature can create an imbalance within the photosynthetic apparatus if some processes slow more than others (Sage and Kubien, 2007). Most plants in temperate climates encounter suboptimal temperatures at some point in the growing season (Sage and Kubien, 2007). Plants that are well-adapted to seasonal climates have evolved ways to alleviate low temperature constraints on metabolism by changing relative protein levels, or their activities, to compensate for slower reaction times, or by simply increasing total leaf protein (Way and Sage, 2008b; Atkin and Tjoelker, 2003).

$C_3$ photosynthesis is usually studied in terms of three rate-limiting capacities (Sharkey, 1985). The first is the capacity of Rubisco to carboxylate ribulose-1,5-bisphosphate (RuBP), the second is the capacity of electron transport and the Calvin cycle to regenerate RuBP, and the third is the capacity of starch and sucrose synthesis to utilize triose-phosphates and liberate $P_i$ (Sharkey, 1985; Sage and Kubien, 2007). At ambient CO$_2$ levels and saturating light, $C_3$ photosynthesis is often limited at low temperatures by $P_i$-regeneration, particularly in warm-climate plants (Sage and Sharkey, 1987; Sage and Kubien, 2007). For example, below 15 °C, $P_i$-regeneration limits photosynthesis in both grapevine and sweet potato (Hendrickson et al., 2004b; Cen and Sage, 2005). Plants that can acclimate to low temperatures, such as Arabidopsis and winter cereals, generally increase the level of available $P_i$ by increasing the activity of enzymes responsible for sucrose and starch synthesis (Strand et al., 1999; Leegood and Furbank, 1986; Sage and
Kubien, 2007). Increased enzyme activity is accomplished by higher protein content, and higher expression of sucrose synthesis enzymes relative to total leaf protein (Stitt and Hurry, 2002). Full alleviation of $P_i$-limitations seems to only occur in leaves that develop at cool temperatures (Ikeda et al., 1993; Strand et al., 1999; Stitt and Hurry, 2002). In terms of the temperature response of photosynthesis, acclimation to low temperature by increasing the $P_i$-regeneration capacity would increase $A_{\text{net}}$ at low measurement temperatures (Sage and Kubien, 2007; Berry and Bjorkman, 1980).

In plants that can acclimate to cool temperatures, $A_{\text{net}}$ can also be enhanced by modifications to the capacities for Rubisco carboxylation and RuBP regeneration (Sage and Kubien, 2007). For example, spinach leaves that developed at low temperature had increased RuBP-regeneration capacity, increased Rubisco content, and temperature optimum shifted towards the growth temperature (Yamori et al., 2005). RuBP-regeneration capacity can be increased by changing the composition and fluidity of the thylakoid membrane, which affects diffusion of proteins in the electron transport (Öquist, 1982), or by increasing the amounts of proteins involved in electron transport (Huner et al., 1993). Some plants, such as spinach, can also increase carboxylation rates by increasing the catalytic efficiency of Rubisco (Sage, 2002; Yamori et al., 2006). Such changes are costly, requiring larger nitrogen investments per leaf area and larger cell volumes to accommodate higher protein contents (Strand et al., 1999; Yamori et al., 2005). A substantial shift in temperature into a range where $A_{\text{net}}$ is depressed is therefore usually required to induce cold acclimation (Sage and Kubien, 2007).

1.3.4 The balance between photosynthesis and respiration

The net CO$_2$ assimilation rate reflects the balance between photosynthesis ($P$) and respiration ($R$). In many species, the ratio of $R$ to $P$ is constant over a range of moderate temperatures, reflecting the interdependence of these two processes for substrates (Loveys et al., 2002; Atkin et al., 2005). At low temperatures, this balance may break down be-
cause $R$ and $P$ have different temperature responses and acclimation potentials (Atkin et al., 2005). In general, the short-term response of respiration is to increase exponentially with measurement temperature. The steepness of the increase ($Q_{10}$) depends on the acclimation state. The $Q_{10}$ of leaf $R$ declines with increases in (short-term) measurement temperature across species, so at a given acclimation state, changes in $R$ are greatest at low temperatures (Atkin and Tjoelker, 2003). If the temperature change persists for more than one day, $R$ may begin to acclimate (Atkin and Tjoelker, 2003; Atkin et al., 2005). Acclimation to cool temperatures usually involves increases in $R$. This is accomplished by changing the activation state of enzymes, increasing the availability of substrates like soluble carbohydrates and ADP, increasing mitochondrial protein relative to total leaf protein, or by changing the relative amounts of specific enzymes (Atkin et al., 2005; Atkin and Tjoelker, 2003). Numerous studies have attempted to find general patterns associating $R$ with plant functional type, species thermal origin, or species inherent growth rate, but no convincing patterns have yet emerged (Atkin et al., 2005; Campbell et al., 2007). Unlike photosynthesis, $R$ acclimates in most species (Atkin and Tjoelker, 2003; Atkin et al., 2005; Way and Oren, 2010). Species that can maintain homeostasis of net CO$_2$ assimilation rates at cooler temperatures usually adjust both $R$ and $P$.

### 1.4 Thesis objectives

The objective of this thesis is to determine the role of low temperatures in determining the northern range limit of kudzu (*Pueraria montana* var. *lobata*). My approach to this problem is to focus on how low temperatures might restrict kudzu performance or establishment at important life history stages. My emphasis is therefore on relating kudzu’s physiological response to low temperature to conditions experienced at its range limit. I have designed my experiments with the modeler in mind, seeking where possible to sample broadly, identify thresholds, and characterize uncertainty (Woodward and

In keeping with the emphasis on life history stages, I have organized my thesis around the four seasons. In Chapter 2, I establish a correlation between cold winters and kudzu’s northern range limit, and look at whether freezing mortality explains that correlation. In Chapter 3, I evaluate whether chilling temperatures in the spring will hinder kudzu canopy development and photosynthesis. In Chapter 4, I determine the temperature response of photosynthesis and growth during June and July and evaluate whether kudzu might have the potential to acclimate to cool growth temperatures that may occur mid-summer. In Chapter 5, I look at how growth and photosynthesis respond to autumn conditions.

Chapter 2: Winter cold tolerance of kudzu (*Pueraria montana* var. *lobata*) and its significance for future range expansion. The objectives of this chapter are two-fold. First, I set out to confirm if kudzu’s distribution has tracked the northward movement of minimum winter temperatures. With collaborators, I then modeled future range expansion into northern climate zones. Correlations are often used to infer future range shifts, but the assumed underlying mechanisms are seldom tested. Thus, my second objective is to determine whether survival of winter freezing temperatures could explain this correlation.

Chapter 3: Springtime chilling of kudzu (*Pueraria montana* var. *lobata*) and its significance for future range expansion. The objective of this chapter is to evaluate whether chilling temperatures in the spring pose a barrier to kudzu establishment in northern climates. To do this I described kudzu growth and photosynthesis responses in the field during a cool but mild year, and during a year with a severe chill. I establish lower threshold temperatures for kudzu growth and photosynthesis, which are important for evaluating where kudzu might be able to establish and prosper.
Chapter 4: The temperature response of growth and photosynthesis in kudzu *(Pueraria montana* var. *lobata)* and its significance for future range expansion. In this chapter, I evaluate whether cool summer temperatures will curtail rapid vegetative growth of kudzu. A main objective of this chapter is to describe the temperature response of photosynthesis and growth in a field setting at its northern distribution limit. Since acclimation could alleviate low temperature restrictions and allow kudzu to perform better in cool conditions than a temperature response curve alone would indicate, I also compared the temperature response of kudzu growing at cool and warm temperatures using a combination of lab and field experiments.

Chapter 5: Autumn frost tolerance of kudzu *(Pueraria montana* var. *lobata)* and its significance for future range expansion. If photosynthesis continues after growth ceases in autumn, then kudzu might be storing extra carbon for the winter (Chapin et al., 1990). Thus, the objective of this chapter is to determine the temperatures associated with the onset of autumn dormancy, leaf death, and the ramping down of photosynthesis. To do this I observed leaf physiology in potted plants over three summer-to-fall transitions. These data will help determine temperatures associated with the end of the growing season performance, when photosynthate is directed to cold acclimation and storage rather than growth.

I have organized my thesis into these four self-contained data chapters because it emphasizes how I have thought about the problem. It also permits me to include the important descriptive work I have done in the process of developing this system.
Chapter 2

Winter cold tolerance of kudzu
(Pueraria montana var. lobata) and its significance for future range expansion

This chapter is the product of collaborations with Rowan F. Sage, Katharine Hayhoe, Jeff van Dorn and Lewis Ziska. Rowan Sage contributed ideas and helped with the writing. Katharine Hayhoe and Jeff van Dorn did the climate modeling, and Lewis H. Ziska contributed data (noted in the figure captions).

2.1 Abstract

Kudzu is a notorious invasive vine in North America that has migrated northward as winters have warmed. The biogeographic correlation between kudzu’s northern range limit and the −20°C isocline for mean minimum winter air temperature was confirmed using an updated distribution map and down-scaled climate models. These isoclines were
modeled based on future warmer climate scenarios to illustrate kudzu’s potential future distribution. The implied mechanism controlling kudzu’s northern limit is that kudzu cannot survive winter temperatures colder than those at its current range limit. This hypothesis was evaluated with electrolyte leakage assays on winter-dormant stems collected from fourteen populations throughout kudzu’s naturalized range. To test for acclimation to winter cold, the critical temperature of fully-acclimated aboveground stems, belowground stems, and roots collected at a recently discovered kudzu population in southern Canada was also determined. Aboveground stems collected in southern Canada survived to $-27^\circ C$, while belowground stems survived to $-17^\circ C$, which is colder than minimum soil temperatures at the current range limit. There is no evidence that freezing mortality explains the biogeographic correlation. This result implies that the model of kudzu’s future distribution either correctly estimates (because kudzu is limited by a correlate of low temperature), or underestimates (because kudzu is not limited by low temperature), kudzu’s future distribution. This result should help land managers assess and mitigate the risk of kudzu invasion into southern Canada.

2.2 Introduction

Species distributions are shifting poleward and upward in response to anthropogenic climate change (Parmesan and Yohe, 2003; Walther et al., 2005). Invasive species are no exception (Walther, 2007). Many harmful introduced plant species are poised to respond quickly to a changing climate, especially those with strong CO$_2$ responses, fast growth rates, unimpeded dispersal, and few biotic limitations (Dukes and Mooney, 1999; Walther et al., 2009). If the range limits of these species are constrained by cold temperatures, then they should be able to expand into new habitat made available by global warming (Walther, 2003a). This could exacerbate existing problems associated with climate change, like declines in regional biodiversity and water quality (MEA, 2005). It is there-
fore a high priority to be able to predict, manage, and potentially mitigate future invasion problems in the context of climate change.

Models of species distributions are valuable tools for biodiversity management (Naylor, 2000). Species distribution modeling has made large advances in recent years, but it is still a challenge to predict invasive species distributions (Thuiller et al., 2005; Gallien et al., 2010). A key assumption of most modeling methods is that species are in equilibrium with climate (Guisan and Zimmermann, 2000). Broad biogeographic patterns support this assumption (von Humboldt and Bonpland, 1807; Schimper and von Faber, 1935; Polunin, 1960) but at a finer scale, range limits often fall short of the fundamental climatic niche (Colwell and Rangel, 2009; Pulliam, 2000; Hutchinson, 1957). For native species, a range of biotic, edaphic, historical, and stochastic factors could be at play (Gaston, 2009; Colwell and Rangel, 2009), while recently introduced species may not have had enough time to disperse into all of their suitable habitat (Vaclavik and Meentemeyer, 2009; Monahan, 2009). Also, introduced species may occupy a different climate space in their adopted range than that of their native range. This might occur if they experience a novel suite of biotic interactions, or if they undergo rapid local adaptation (Gallien et al., 2010; Alexander and Edwards, 2010). The empirical testing of assumptions associated with biogeographic patterns is one way to improve predictions of future species distributions.

Kudzu (Pueraria montana var. lobata (Willd.) Ohwi) is an Asiatic semi-woody vine that was introduced to eastern North America in the late 19th century (Forseth and Innis, 2004). Originally promoted as an ornamental, it was soon grown for forage and for the conservation and rehabilitation of soils (Winberry and Jones, 1973). Kudzu infestations degrade air quality with isoprene emitted from leaves and with nitric oxide emitted from soils, but their primary ecological effect is to reduce biodiversity through light competition (Sharkey and Loreto, 1993; Forseth and Innis, 2004; Hickman et al., 2010). Kudzu has earned its position as the only plant on Time magazine’s “Top 10
Worst Invasive Species” list (Friedman, 2010) for its unparalleled ability to cover fields, vehicles, and buildings with impenetrable foliage (Blaustein, 2001). Kudzu inspires such loathing that it is a go-to example for scholars interested in how people talk about invasive species (Eskridge, 2010). It is even a metaphor for a malevolent thing that cannot be killed (Rothstein, 1996). These attitudes form a stark contrast to kudzu’s previous portrayal as the “Savior of the South” (Alderman, 2004). In the early 20th century, kudzu was one of the few crops that could still flourish on the cotton-gutted soils in the southern U.S. states (Winberry and Jones, 1973; Alderman, 2004). Kudzu’s efficacy as a soil stabilizing crop led U.S. government agencies to plant it throughout the southeastern states in the early- to mid-twentieth century, thereby accelerating kudzu’s range expansion in North America (Winberry and Jones, 1973; Forseth and Innis, 2004). By the mid-1950’s kudzu’s drawbacks outweighed the benefits, and kudzu became known as a noxious weed (Blaustein, 2001).

Proponents of kudzu identified cold winters as a principal limit to establishment in northern areas (Bailey, 1939; Shurtleff and Aoyagi, 1985). This impression is supported by a correlation between the northern range limit and the −20°C isocline for mean minimum air temperature (Fig. 2.1A) (Sasek and Strain, 1990). In recent decades, winters have warmed concurrently with the appearance of kudzu beyond its described northern range limit (Sasek and Strain, 1990), providing further evidence that cold temperatures limit northward range expansion. This correlation has been a key component of speculations about, and models of, how kudzu’s distribution might change in future climates (Sasek and Strain, 1990; Follak, 2011; Ziska et al., 2011; Wolfe et al., 2008; Jarnevich and Stohlgren, 2009). The implied and oft-stated—but untested—mechanism behind this correlation is that kudzu cannot survive cold winters (Forseth and Innis, 2004). The utility of the published predictive models for management decisions depends in part on whether freezing temperatures are in fact range-limiting. Here, we evaluate whether cold winters determine kudzu’s northern distribution limit by testing the hypothesis that
−20°C corresponds to a lethal threshold.

2.3 Methods and Materials

2.3.1 Kudzu distribution in North America

Reports that kudzu has migrated northward since 1970 were confirmed by consulting plant distribution databases, herbaria, government agencies, and local experts, focusing on kudzu’s distribution in U.S. states north of 39°N and east of 96°W, and in Ontario, Canada. The northernmost populations were visited in August 2009 to control for misidentifications. The 957 kudzu locations and their references have been deposited in the publically available Early Detection and Distribution Mapping System database (www.eddmaps.com) at the University of Georgia (Bargeron, 2009).

2.3.2 Climate modeling

The minimum temperature that corresponds to the current northern range limit of kudzu was determined by simulating the historical and projected change in the isoclines representing 0–2 days at or below −20°C as well as 0–2 days at or below −22°C and −18°C. The criterion of 0–2 days was chosen because it represents a single short excursion to an extreme temperature in an otherwise milder winter. The isoclines at −22°C and −18°C attempt to account for some of the microclimatic variation not captured by the regional-scale climate data (Jones, 1992; Helmuth et al., 2010). If this brief exposure is sufficient to cause kudzu mortality, then changes in the future location of these isoclines should explain the future northern range limit of kudzu. The Coupled Model Intercomparison Project’s “20th Century Climate in Coupled Models” or 20C3M scenarios (Covey et al., 2003) were used to simulate observed climate over the past century. Projections of future climate change were based on the A2 (mid-high) and B1 (lower) emission scenarios from the “Intergovernmental Panel on Climate Changes Special Report on Emission Sce-
narios” (Nakicenovic et al., 2000). The coarse-scale results of simulations by 16 of the atmosphere-ocean general circulation models (AOGCMs) corresponding to the IPCCs 20C3M, A2, and B1 scenarios were statistically downscaled to daily values across the continental U.S. and southern Canada with a resolution of 1/8th degrees (IPCC, 2007). This well-established downscaling approach uses an empirical statistical technique that maps the probability density functions for modeled monthly and daily precipitation and temperature for each climatological period onto those of gridded historical observed data, so the mean and variability of both monthly and daily observations are reproduced by the climate model outputs (Maurer et al., 2002). This method compares favorably to regional climate model simulations (Wood et al., 2004) and was used as the basis for the regional climate projections in the 2009 U.S. Global Change Research Program report, “Global Climate Change Impacts in the United States” (U.S. Global Change Research Program, 2009).

2.3.3 Plant material

To determine the cold temperature threshold that causes lethal cell damage (critical temperature), winter-dormant stems were collected in March 2007 at seven northern and seven southern populations in kudzu’s naturalized range (Table 2.1). Stem segments with one healthy node were sampled 1–2 m above ground at 2–3 m intervals. Kudzu reproduces vegetatively when trailing stems take root at a node (Miller and Edwards, 1983). Rooted nodes become independent crowns that eventually become buried in soil along with their connecting internodes. To test the cold temperature threshold of crowns, belowground stems were also collected at one site in Beltsville, Maryland. Material was stored in soil at 4 °C until it was evaluated for freezing tolerance.

To test for acclimation to winter cold, the critical temperature of above- and belowground stems in plant material collected at a recently discovered kudzu site in Leamington, Ontario (42.02°N, 82.74°W) was also determined. The kudzu stand was sampled
Table 2.1 – Kudzu populations for March 2007 collections, arranged by increasing latitude and divided into southern and northern groups (Fig. 2.4) All but the three southernmost sites are shown as black dots in Fig. 2.1B

<table>
<thead>
<tr>
<th>Code</th>
<th>Site description</th>
<th>Latitude, °N</th>
<th>Longitude, °W</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS2</td>
<td>Roadside, Hwy 61 S of Vicksburg, MS</td>
<td>32°5′34.8″</td>
<td>90°55′30.0″</td>
</tr>
<tr>
<td>GA2</td>
<td>Railway embankment, Statesboro, GA</td>
<td>32°26′17.3″</td>
<td>81°46′49.7″</td>
</tr>
<tr>
<td>GA3</td>
<td>Riverbank, Athens, GA</td>
<td>33°55′55.8″</td>
<td>83°55′55.8″</td>
</tr>
<tr>
<td>MS1</td>
<td>Roadside, Hwy 7 S of Holly Springs, MS</td>
<td>34°40′54.4″</td>
<td>89°27′42.2″</td>
</tr>
<tr>
<td>SC</td>
<td>Roadside, Hwy 123, Greenville, SC</td>
<td>34°49′29.4″</td>
<td>82°18′29.1″</td>
</tr>
<tr>
<td>NC1</td>
<td>Roadside, Hwy 73, Charlotte, NC</td>
<td>35°27′5.8″</td>
<td>80°47′56.9″</td>
</tr>
<tr>
<td>TN</td>
<td>Narrows of the Harpeth State Park, TN</td>
<td>36°8′57.4″</td>
<td>87°6′53.7″</td>
</tr>
<tr>
<td>VA</td>
<td>Grounds, St. Pauls Episcopal Church, Arnolds Corner, VA</td>
<td>38°16′33.8″</td>
<td>77°12′14.0″</td>
</tr>
<tr>
<td>MD1</td>
<td>Forest edge, USDA Beltsville, MD</td>
<td>39°0′13.9″</td>
<td>76°51′3.2″</td>
</tr>
<tr>
<td>MD2</td>
<td>Roadside, McKee Beshers Wildlife Management Area, MD</td>
<td>39°5′4.2″</td>
<td>77°25′17.7″</td>
</tr>
<tr>
<td>DE1</td>
<td>Roadside, Rte 13 at Garrison Lake, DE</td>
<td>39°14′32.8″</td>
<td>75°35′11.5″</td>
</tr>
<tr>
<td>DE2</td>
<td>Railway embankment, Wilmington, DE</td>
<td>39°43′13.8″</td>
<td>75°34′50.9″</td>
</tr>
<tr>
<td>PA2</td>
<td>Abandoned gas station, Philadelphia, PA</td>
<td>40°3′9.3″</td>
<td>75°14′10.8″</td>
</tr>
<tr>
<td>NY</td>
<td>City sump, Huntington Station, NY</td>
<td>40°48′37.3″</td>
<td>73°24′34.9″</td>
</tr>
</tbody>
</table>
four times between November 2009 and May 2010 to track changes in winter cold tolerance (acclimation) in response to winter cold. The Leamington site is on a steep south-facing embankment on the north shore of Lake Erie. It is approximately 120 × 50 m in size and 7 to 40 years old. The site is bordered to the east and west by forest, to the south by the lake, and to the north by a plowed field. Sampling occurred along two parallel east-west transects that ran the length of the patch 3 m from the north and south edges. For belowground material, shallow holes were dug every 2–3 m along each transect. For aboveground material, 1-y-old unrooted stems were sampled every 2–3 m along each transect. In February, excavated frozen soil was thawed just enough to remove the tissue. Harvested belowground samples were placed in bins filled with native soil. Aboveground samples were placed on the soil and covered with kudzu leaf litter. Samples were transported immediately to the lab (5 hours) and stored outside (Nov, Feb, Mar) or at 4°C (May) to minimize deacclimation. Freezing tests were completed within two weeks of collection.

2.3.4 Environmental conditions

Air temperatures and snow cover at Leamington were recorded by the Environment Canada weather station in Harrow, ON, Canada, ca. 20 km west of the kudzu population (Harrow CDA, 42.03°N, 82.9°W, elevation = 191 m.a.s.l., Climate ID = 6133362, WMO ID = 71298, TC ID = XHA) (www.climate.weatheroffice.gc.ca). Soil temperatures were measured with thermistors (HOBO U23 Pro v.2, Onset Computer Corp, Pocasset, MA, USA) placed 5 cm deep in soil beneath the kudzu canopy at three locations: near the lake, mid-slope, and at the top of the slope near the plowed field. The values were similar, so only the mid-slope values are reported.
2.3.5 Freezing tests

In 2007, whole stems were frozen in a programmable freezer (Model 2800, Thermotron Industries, Holland MI, USA). Freezer temperature was lowered to the target temperature at 8 °C h⁻¹, maintained for 4 hours, then raised at 16 °C h⁻¹. Target temperatures were 4 °C (control), −4°, −8°, −12°, −16°, −20°, −24°, and −30 °C. Both tissue damage and survival were assessed for each stem. Tissue damage was assessed by equilibrating a small segment of the internode (5–10 mm) in 20 ml ddH₂O at 20–22 °C. This allows the ionic contents of cells damaged by the treatment to leak into solution. The electrical conductivity of the solution was measured with a hand-held conductivity meter (Ultrameter 4P, Myron L. Company, Carlsbad, CA, USA). The samples were then boiled to kill all the cells, re-equilibrated in the same solution, and the total conductivity was measured. The proportion of cells damaged by the freezing treatment is the ratio of treatment conductivity to total conductivity, or relative conductivity (RC) (Hallam and Tibbits, 1988). Artificial nucleation with ice crystals and slower cooling rates (1 °C h⁻¹) did not alter the results (Appendix B).

To assess survival of stem buds following the freezing treatment, samples were planted in 0.1 L pots in a glasshouse (16/8 h, 26°/18 °C day/night, ≥ 1000 µmol⁻¹ m⁻² s⁻¹ light). Stems were half-buried at a 45° angle in soil (20% Triple-mix, 50% Pro-mix, 10% perlite, 20% sand) with the bud just beneath the soil surface (Gusta, 2003). Stems were scored as “survived” if the germinating bud broke the soil surface. Temperature treatments were randomized within each of two replicate trials. Roots do not generate shoots, so root survival was not assessed.

In 2009–2010, whole stems and roots from Leamington were artificially frozen to at least eight temperatures between 4 °C (control) and −34 °C in one of three programmable freezers (one Model 2800 and two Model S-16-8200, Thermotron Industries, Holland MI, USA). The target temperatures for each tissue type by month combination were selected to optimally sample the expected relative conductivity response. Target temperatures
were randomly ordered. Sample sizes varied from 3–17 for each sample point. Based on
the results from 2007, larger sample sizes were used for temperatures near the hypothe-
sized critical temperature of $-20^\circ C$, except for roots in February ($N=1–3$) when frozen
soils limited the collection of intact roots. Relative conductivity was assessed following
each freezing treatment.

Critical temperature was determined in a two-step process that links treatment tem-
perature to survival via relative conductivity (RC). First, the lethal level of cell damage
was determined by fitting a generalized linear model following a binomial distribution
to the survival data from 2007. Stem RC was the sole predictor, and a non-canonical
clog-log link function was used to accommodate the greater number of “successes” (killed
stems) than failures (Hardin and Hilbe, 2007). The model link function was solved nu-
merically to find the threshold level of RC that results in 99.7% probability of death.

Next, the temperature that results in the threshold RC was determined by fitting the
proportional RC data with a second binomial glm (logit link). The explanatory variables
were treatment temperature and collection site location, which was best parameterized
by a variable that divided the fourteen populations into northern and southern groups
(“Region”) (Table 2.1). The logit was solved numerically to determine the critical tem-
perature for each population. This was done because RC was a better predictor of
survival (explaining 43% of the total deviance) than treatment temperature (explaining
27% of the total deviance). R Project software v. 2.8.1 (R Development Core Team,
2011) was used for all analyses, and all models were evaluated for conformity to assump-
tions of parametric analyses (Faraway, 2006; Zuur et al., 2009). Non-significant terms
were retained if they improved model diagnostics.

In March 2010, the freezing experiment was repeated with a fresh set of aboveground
stems collected in Leamington. The goal of this study was to give the tissue time to
depend its acclimation state by exposing it for longer periods of time to non-damaging
freezing temperatures. To do this the stems were allowed to incubate for 9–10 hours
Figure 2.1 – The distribution of kudzu in North America and contours representing 0–2 days per year below $-20 ^\circ C$. (A) Kudzu distribution limit in 1971 redrawn from Sasek and Strain (1990) (green line) with contours for the 20 year period 1951–1970, and (B) kudzu occurrences in 2009 derived from herbarium records, weed databases, and direct observation (green triangles), with contours for the 20 year period 1981–2000 (medium blue). The contours for 0–2 days per year below $-18 ^\circ C$ (pale blue) and $-22 ^\circ C$ (dark blue) are also shown (Fig 2B). Black dots show the 11 northernmost sites visited in the March 2007 collection (Table 2.1). K. Hayhoe and J. van Dorn did the climate modeling and made the maps in collaboration with H.A. Coiner.
every 4–5°C before further cooling (1°C h\(^{-1}\)) to \(-18^\circ\), \(-20^\circ\), \(-24^\circ\), \(-28^\circ\), \(-32^\circ\), and \(-36^\circ\) C. Stems were thawed in a second freezer at 1°C h\(^{-1}\) and relative conductivity was measured.

### 2.3.6 Seed survival

Seeds were collected from Montrose, New York and Beltsville, Maryland in winter 2006, and from Huntington, New York in fall 2007. To simulate the effect of a deep mid-winter freeze on seed germination, replicated subsamples of 9–20 seeds were subjected to artificial freezing treatments in a programmable freezer (Revco Ultima 2, Thermo Electron Corp., Asheville NC USA) in January 2008. Temperatures were lowered by 6°C over 30 min and held constant for 24 hours before lowering the temperature again. The following spring, seeds were planted in 100% Pro-mix in a glasshouse (50% daylight, 20°C) and germination was monitored for 74 days. Each temperature treatment was replicated three times for a total of 27–60 seeds per population per temperature.

### 2.4 Results

#### 2.4.1 Past and future distribution of kudzu in North America

Kudzu has moved north since 1971 (Fig. 2.1). In 1971, the U.S. Department of Agriculture reported that kudzu was confined to southeastern Missouri, the southern tips of Illinois and Indiana, and southern Pennsylvania and Delaware. Kudzu was not reported in Ohio or in western Pennsylvania, nor was it in New York or the New England states (Fig. 2.1A) (U.S. Department of Agriculture, 2010; Sasek and Strain, 1990). In 2009, kudzu was found to be common in New York City, throughout Delaware, and in the southern half of Pennsylvania, Ohio, Indiana, Illinois, and Missouri (Fig. 2.1B). Isolated populations were found further north in maritime Connecticut and Massachusetts, in Albany (New York) and in the Hudson River Valley (New York). In the midwest, kudzu
Figure 2.2 – Climate-based projections of the future location of contours representing 0–2 days under $-20^\circ$C, along with contours representing 0–2 days at $-18^\circ$C and $-22^\circ$C. The left-hand panels represent projected locations of these isoclines under the A2 mid-high emissions scenario, while the right-hand panels use the B1 lower emissions scenario. K. Hayhoe and J. van Dorn did the climate modeling and made the maps in collaboration with H.A. Coiner.
is now found in Cleveland (Ohio), northern Indiana, Chicago (Illinois), and along the eastern shore of Lake Michigan (Fig. 2.1B). The first documented Canadian population is on the north shore of Lake Erie, near Leamington, Ontario.

This range shift corresponds to winter warming over the same period. Isoclines that represent 0–2 days where the daily minimum temperature was −20 °C or colder were further north in 1981–2000 than they were in 1951–1970 (Fig. 2.1). The similarity between the shift in kudzu’s northern limit and the shift in the −20 °C isocline is most apparent in southern Illinois and southern Indiana (Fig. 2.1). The congruent changes in both the −20 °C isocline and the northern range limit of kudzu support the notion that low winter temperature limits kudzu’s range expansion to the north (Sasek and Strain, 1990; Follak, 2011; Ziska et al., 2011; Wolfe et al., 2008; Jarnevich and Stohlgren, 2009).

If this correlation has a temperature-based mechanism, then the future location of these isoclines represents the potential for kudzu range expansion under climate change. Under the higher emissions scenario (A2), model projections of the future −20 °C isocline show that in the next 10–30 years, kudzu should be able to survive winter in large portions of the Great Lakes region, including much of southern Michigan and most of the north shore of Lake Erie (Fig. 2.2). By 2069, this area will have expanded to include most of the Midwestern states, lake- and maritime-influenced New York, and most of southern New England. In Canada, most of southwestern Ontario should be habitable by kudzu. By the end of this century, winter temperatures will be mild enough for kudzu to survive on all of the Great Lakes shorelines except for the north shores of Lakes Huron and Superior. Under the lower emissions scenario (B1, Fig. 2.2), the newly habitable areas are qualitatively similar to the higher emissions scenario. The largest differences are that north-central Pennsylvania, the mountains in West Virginia, western New York and southern Maine will remain too cold for kudzu under the lower emissions scenario (Fig. 2.2).
Table 2.2 – Number of kudzu stems that survived low temperature treatments. A stem was scored as survived if a bud broke the soil surface. Temp is the minimum exposure temperature in freezing tests, while North and South are the geographic groups of above-ground stems. Crowns are belowground stems from Beltsville, MD. The number survived out of the total is given along with % survival in parentheses.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Trial 1</th>
<th></th>
<th>Trial 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temp, °C</td>
<td>North</td>
<td>South</td>
<td>Crowns</td>
</tr>
<tr>
<td></td>
<td>+4</td>
<td>9/14 (64%)</td>
<td>6/14 (43%)</td>
<td>1/1 (100%)</td>
</tr>
<tr>
<td></td>
<td>−4</td>
<td>4/5 (80%)</td>
<td>5/7 (71%)</td>
<td>1/1 (100%)</td>
</tr>
<tr>
<td></td>
<td>−8</td>
<td>4/7 (57%)</td>
<td>3/7 (43%)</td>
<td>1/1 (100%)</td>
</tr>
<tr>
<td></td>
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<td>6/7 (86%)</td>
<td>1/7 (14%)</td>
<td>0/1 (0%)</td>
</tr>
<tr>
<td></td>
<td>−16</td>
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</tr>
<tr>
<td></td>
<td>−30</td>
<td>0/7 (0%)</td>
<td>0/7 (0%)</td>
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</tr>
</tbody>
</table>
2.4.2 Low temperature mortality in northern and southern populations

The threshold amount of cell damage that prevents kudzu survival was determined in winter-dormant kudzu stems collected at 14 southern and northern populations in March 2007 (Table 2.1). In this portion of the study, no above- or belowground stem survived at $-20\,^\circ\text{C}$ or below. Only aboveground stems from northern populations survived at $-16\,^\circ\text{C}$ (Table 2.2). Southern stems and belowground stems from Maryland survived treatment to $-12\,^\circ\text{C}$, but not below. Stem relative conductivity (RC) began increasing with decreasing temperature below $-10\,^\circ\text{C}$ (Figs. 2.3B, B.1), reflecting increased tissue damage due to cold temperatures. The generalized linear model (glm) of RC fit to survival was not improved by adding in geographic or tissue type information (model results not

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Figure 2.3 (following page) – Relationship between treatment temperature, relative conductivity (RC) and survival. (A) Probability of stem death as a function of RC for pooled above- and belowground stems collected from the 14 populations sampled in 2007 (Table 2.1). The solid black line is the generalized linear model (glm) Survival = RC (Table B.1), with its dashed 95% CI. The vertical dotted line at 36% RC shows the point at which the probability of death is 99.7%. (B) The temperature response of relative conductivity for the same aboveground stems in panel A. Datapoints are means ± se ($N = 7–21$) of the seven northern (squares) and the seven southern populations (circles) from Table 1. Data from both geographic regions are fit simultaneously with the glm $\text{RC} = \text{Temp} + \text{Region} + \text{Temp:Region} + \text{Temp}^2$ (solid line, Table B.2) and its dashed 95% CI, where “:” indicates that a stem’s temperature response depends on its geographic origin. The vertical dotted lines at $-19.6\,^\circ\text{C}$ and $-14.4\,^\circ\text{C}$ show the critical temperature for survival of each geographic group. (C) The temperature response of relative conductivity for the thirteen belowground stems collected in Beltsville, Maryland. The vertical dotted line at $-13.4\,^\circ\text{C}$ shows the critical temperature for survival for belowground stems.
Chapter 2. Winter freezing tolerance

A

Probability of death

0.0 0.2 0.4 0.6 0.8 1.0

0.0 0.2 0.4 0.6 0.8 1.0

Relative conductivity

0.0 0.2 0.4 0.6 0.8 1.0

B

Treatment temperature (°C)

-30 -25 -20 -15 -10 -5 0 5

Relative conductivity

0.0 0.2 0.4 0.6 0.8 1.0

C

Relative conductivity

0.0 0.2 0.4 0.6 0.8 1.0

-30 -25 -20 -15 -10 -5 0 5

Treatment temperature (°C)
shown), indicating that stems from all populations die at the same RC. Belowground stem survival did not differ from aboveground stem survival ("Stem type" main effect (all populations pooled), \( P(<| \chi^2 |\nu=1.244) = 0.11 \)), so the RC that results in a 99.7% probability of death in stems from all populations is 36% (Fig. 2.3 A).

This threshold RC was related to treatment temperature using a second glm. This model described 92% of the variation in relative conductivity, with temperature alone accounting for 73% of the deviance (Fig. 2.3 B, Appendix B Table B.2). The balance of the explained deviance is accounted for by non-linearity in the response ("Temp^2", 12%, \( P(<| \chi^2 |\nu=1.228) = 0.003 \)) and the significant difference between northern and southern regions ("Region", 5%, \( P(<| \chi^2 |\nu=1.229) = 0.049 \)) (Table B.2). That difference is apparent in how the critical temperatures of the 14 populations cluster when plotted against latitude (Fig. 2.4). The mean critical temperature of the seven southern populations was \(-14.4^\circ\text{C}\) with a 95% CI of \([-10.6, -17.7]^\circ\text{C}\), while that of the seven northern populations was \(-19.6^\circ\text{C}\) with a 95% CI of \([-16.1, -23.2]^\circ\text{C}\) (Fig. 2.4). Belowground stems from Beltsville, Maryland showed an RC response to temperature similar to that of aboveground stems (Fig. 2.3, Table B.3). The critical temperature of crowns was \(-13.4^\circ\text{C}\), but there is substantial uncertainty in this estimate because of the small sample size (\(N = 13\), 95% C.I.=[> 4, -21.4]^\circ\text{C}\).

### 2.4.3 Acclimation to freezing temperatures

To test for deeper freezing tolerance associated with acclimation to freezing temperatures, roots and above- and belowground stems were sampled at the kudzu population in Leamington, Ontario from Nov 2009 to May 2010 (Figs. 2.5, B.2, B.3). That winter, air temperatures declined to an extreme minimum of \(-17.9^\circ\text{C}\) in early January 2010 (Fig. 2.6 A). The average winter (December to February) air temperature was \(-3.3^\circ\text{C}\), while the average minimum air temperature over the three winter months was \(-6.2^\circ\text{C}\). Soil temperature logged at 5 cm depth underneath the kudzu canopy reached an extreme
Figure 2.4 – The critical temperature associated with the relative conductivity that results in 99.7% probability of death for each of the fourteen kudzu populations (Figs. B.1, 2.3 A). The glm $RC = Temp + Temp^2$ was fit to each population individually. Points are the numerical estimates of critical temperature plotted against latitude of the population (Table 2.1), while the whiskers extend to the edges of the 95% CI for that population. The mean critical temperature of the seven southern populations ($-15.1 ^\circ C$, 95% C.I.$=[-12.8, -16.9] ^\circ C$) is significantly warmer than that of the seven northern populations ($-19.7 ^\circ C$, 95% C.I.$=[-17.9, -21.4] ^\circ C$) (Table B.2).
minimum of $-2.1\, ^\circ C$ in early February (Fig. 2.6 A), with a winter average of $0.76\, ^\circ C$. The average minimum soil temperature in the same period was $0.53\, ^\circ C$. Soil temperature remained close to $0\, ^\circ C$ when snow was on the ground (Fig. 2.6A).

The three types of tissue differed in their freezing tolerance. The main effect of treatment temperature ("Temp", $P(<| \chi^2 |_{\nu=1,746}) < 0.001$) explained 29% of the total deviance in the data, while month of collection ("Month", $P(<| \chi^2 |_{\nu=3,741}) < 0.001$) explained another 26% (Table B.4, Figs. 2.5, B.2, B.3). This shows that the mean response of all tissue types changed over the course of the winter, but there was no interaction ("Temp:Month", $P(<| \chi^2 |_{\nu=2,736}) = 70.5$) (Table B.4). The direction of these changes (increased cold tolerance in mid-winter) is consistent with acclimation. Tissue types differed in both their average response to treatment temperature ("Type", $P(<| \chi^2 |_{\nu=2,744}) < 0.001$), and in the shape of that response ("Temp:Type", $P(<| \chi^2 |_{\nu=2,739}) < 0.001$), together accounting for an additional 19% of the deviance (Table B.4). The full model (Figs. 2.5, B.2, B.3) explained 80% of the total deviance in the data (Table B.4).

The critical temperature of the three tissue types collected in Leamington was determined by solving the logit of a glm fit to each tissue type for the temperature that resulted in 36% RC (determined in Section 2.4.2). Aboveground stems from Leamington were more freezing tolerant than belowground stems, while roots were the least tolerant of freezing temperatures (Fig. 2.6B). In February, aboveground stems survived to $-26.8\, ^\circ C$ (Table B.5, Fig. B.2). This critical temperature was qualitatively warmer in November ($-21.7\, ^\circ C$) and in March ($-23.2\, ^\circ C$) (Fig. 2.6, Table B.5). The March critical temperature measured in Leamington ($-23.2\, ^\circ C$) is similar to that measured in March 2007 in the seven northern populations ($-19.6\, ^\circ C$) (Table B.5, Fig. 2.4). In May, when the first shoots were appearing, aboveground stems had lost all freezing tolerance (critical temperature = $1.1\, ^\circ C$) (Table B.5, Fig. B.2). These changes in freezing tolerance in exposed stems are consistent with an ability to acclimate to large fluctuations in air temperatures (Fig. 2.6A).
Figure 2.5 – Relative conductivity (RC) response to temperature in belowground stems collected on (A) Nov 12, 2009, (B) Feb 7, 2010, (C) Mar 21, 2010, and in (D) May 30, 2010 in Leamington, Ontario, Canada (42.02°N, 82.74°W). Points are means ± se of 7–17 samples per temperature. The solid line is the generalized linear model $RC = \text{Temp} + \text{Type} + \text{Month} + \text{Temp:Type} + \text{Temp:Month} + \text{Type:Month} + \text{Month:Temp}^2 + \text{Type:Temp}^2$ (Table B.4) with the 95% CI (dashed lines), where “:” indicates an interaction between variables. The critical temperature shown in Fig. 2.6 is where the lethal 36% RC from Fig. 2.3A (dotted line) intersects the model and the CI.
Figure 2.6 – Environmental conditions and critical temperatures of aboveground stems, belowground stems, and roots collected on Nov 12, 2009, Feb 7, 2010, Mar 21, 2010, and on May 30, 2010 in Leamington, Ontario, Canada (42.02°N, 82.74°W). (A) Minimum daily air temperature at the nearby Harrow, Ontario weather station (grey line) (Climate Data Online, 2010) and daily soil temperature at 5 cm depth (black line) logged every 20 min in the middle of the kudzu patch. Crosses represent days with snow cover. (B) Points are numerical estimates of the critical temperature (see text) of aboveground stems (triangles), belowground stems (circles) and roots (crosses) using the 36% RC survival threshold from Fig 2.3. The maximum extent of the 95% CI is shown as the lower half of a whisker on one of the points.
To check for additional acclimation potential, another set of aboveground stems collected in March 2010 in Leamington were exposed to 9–10 hour incubations at progressively colder temperatures in the lab. The results were compared to the standard ramped method (described in Section 2.3.5); no significant difference in freezing tolerance was found between the methods (as measured by RC) once treatment temperature and its square were accounted for in the glm (“Method” main effect, \( P(\chi^2 | _{1,130}) = 0.146 \)). The interactions between “Method” and “Temp” (and its square) were also insignificant (results not shown).

Belowground stems from Leamington, Ontario tolerated temperatures down to \(-17.0^\circ\text{C}\) (Table B.5, Figs. 2.5, 2.6 B). These stems are typically located in the top 5–10 cm of soil (data not shown), where the temperatures in February 2010 did not go below \(-2.1^\circ\text{C}\) (Fig. 2.4 A). As with the aboveground stems, belowground stems also showed qualitative evidence of deacclimation in March (\(-10.8^\circ\text{C}\)) and in June (\(-2.5^\circ\text{C}\)). In November, the critical temperature of belowground stems (\(-17^\circ\text{C}\)) was similar to that in February (\(-16^\circ\text{C}\)) (Table B.5, Figs. 2.5, 2.6), indicating that belowground stems are already cold acclimated in the fall.

Roots were more sensitive to low temperature than either type of stem (Figs. 2.6 B, B.3, Table B.5). As with the belowground stems, root critical temperature was similar in November (\(-7.9^\circ\text{C}\)) and in February (\(-7.3^\circ\text{C}\)) (Figs. 2.6 B, B.3, Table B.5). Unlike either type of stem, roots tended to retain their freezing tolerance into the beginning of the growing season: in March, the critical temperature was \(-5.6^\circ\text{C}\) and in May the critical temperature was \(-3.4^\circ\text{C}\) (Figs. 2.6 B, B.3, Table B.5).

### 2.4.4 Seed freezing tests

The effect of a deep mid-winter freeze on seed germination was simulated by subjecting seeds to artificial freezing treatments and monitoring germination. Seed germination ranged from 10 to 85%, varying between sites but independent of exposure temperature.
Figure 2.7 – Proportion of seeds that germinated at a range of treatment temperatures. Seeds were collected in 2007 from Beltsville, Maryland (triangles), and in 2006 from Huntington Station, New York (circles) and Montrose, New York (crosses, 41.25°N, 73.94°W) (Table 2.1). L. Ziska contributed the data in this figure.
down to $-36^\circ C$ ("Temp" effect in a binomial glm, $P(<| \chi^2 |_{\nu=1,14}) = 0.088$) (Fig. 2.7). Germination of seeds collected in Beltsville, Maryland was still 60–80% following exposure to $-36^\circ C$ (Fig. 2.7).

2.5 Discussion

The updated map of kudzu occurrence in North America shows that the bulk of North American kudzu populations occur south of the isocline that represents 0–2 days at $-20^\circ C$ or below (Fig. 2.1B). Most of the remaining populations occur south of the $-22^\circ C$ isocline, which is intended to account for some of the uncertainty associated with using large-scale climate data to model local climates (Helmuth et al., 2010). Moreover, the region where kudzu is common has moved northward since 1971, apparently tracking the concurrent northward movement of these isoclines (Fig. 2.1). This is consistent with the common impression in the literature that cold temperatures limit the northern extent of kudzu (Sasek and Strain, 1990; Follak, 2011; Ziska et al., 2011; Wolfe et al., 2008; Jarnevich and Stohlgren, 2009). A natural extension of this result would be to conclude that further warming will cause additional range expansion (Fig. 2.2). This may well happen, but not for the most obvious mechanistic explanation—that winter freezing kills kudzu in areas north of the $-20^\circ C$ isocline.

The results of the freezing experiments do not support the hypothesis that kudzu is killed by exposure to $-20^\circ C$. Fully acclimated above- and belowground stems are likely to survive temperatures colder than the coldest temperatures at kudzu’s current northern range limit. Aboveground stems survive down to $-27^\circ C$ (Figs. 2.6, B.2)—colder than $-20^\circ C$, the mean minimum air temperature at kudzu’s range limit. Absolute minimum temperatures below $-27^\circ C$ would kill exposed stems in a very cold year because stem temperatures are similar to air temperatures in the winter (Table B.6), so in a very cold year, exposed stems would be killed if daily minimum temperatures drop below $-27^\circ C$. 
Stems collected under leaf litter, which insulates against temperature extremes (Sakai and Larcher, 1987), had an RC response no different than that of exposed stems (pooling exposed and duff stems vs. keeping them separate, $P(\chi^2_{1,740} < 0.622) = 0.622$). Stems under litter should therefore be able to survive air temperatures colder than their critical temperature. Belowground stems, which are even more protected from temperature extremes by the thermal capacitance of the soil (Henry, 2007), survived temperatures down to $-17\,^\circ C$ (Figs. 2.5, 2.6). Roots are more sensitive to freezing temperatures, reaching the 36% RC threshold at $-7\,^\circ C$ to $-8\,^\circ C$ (Figs. 2.6, B.3). Kudzu growth in spring depends on carbohydrate stores in roots, but stems also store enough carbohydrates to allow crowns with severed roots to regrow readily (Bailey, 1939, H.A. Coiner, personal observation). Root mortality is therefore unlikely to prevent regeneration in the spring, but it could make kudzu more vulnerable to canopy damage, due *e.g.*, to spring chill, later in the year.

Mortality of belowground stems is unlikely at kudzu’s current range limit because the temperature of frozen soil is much warmer than minimum air temperature (Henry, 2007). At the Leamington site, soil temperatures stayed near the freezing point for most of the winter despite large variations in minimum air temperature (Fig. 2.6 A). At 20 of 31 climatological stations across Canada, soil temperatures did not fall below $-10\,^\circ C$ even though air temperatures were much colder (Henry, 2008). Of those stations where soil temperatures approached $-10\,^\circ C$, the closest to the current northern limit of kudzu was Mirabel, Quebec ($45.67^\circ N, 74.03^\circ W$)—400 km north of the kudzu population in Albany, NY (Fig. 2.1B; Henry, 2008). In Peace River, Alberta ($56.23^\circ N, 117.45^\circ W$), soil temperatures at 5 cm dropped to about $-5\,^\circ C$ when air temperatures were between $-35\,^\circ C$ and $-40\,^\circ C$ (Henry, 2007). Where snowpack is heavier, as in Montmorency, Quebec ($47.32^\circ N, 71.15^\circ W$), soil temperatures stay near 0°C despite air temperatures that exceed $-30\,^\circ C$ (Henry, 2007). Even if aboveground stems were killed by the extreme cold at these locations, the belowground stems would likely survive.
Based on the mechanistic evidence alone, there is a ca. 400 km-wide corridor north of kudzu’s current northern limit where kudzu should be able to survive in the current climate. This corridor includes Vancouver, BC, Ottawa, ON, and other parts of Canada as well as northern U.S. states. Other cool temperate regions, like central Europe, could also be vulnerable to kudzu expansion from established introductions in Italy and Switzerland (European and Mediterranean Plant Protection Organization, 2007; Follak, 2011). Freezing mortality can therefore be ruled out as the mechanism behind the tight correlation between winter minimum temperature and the northern range limit.

2.5.1 Alternative hypotheses for the location of the current range limit

In the absence of mechanistic support for the hypothesis that freezing mortality determines kudzu’s current northern range limit, there are two alternative hypotheses that could explain the location of its distributional limit. The first hypothesis is that kudzu could survive beyond its current northern frontier, but that it has not yet dispersed there. While established kudzu stands expand steadily to “consume” landscapes (Forseth and Innis, 2004; Eskridge, 2010), it is still unclear how kudzu disperses to new locations. Kudzu seeds germinate and develop into vigorous seedlings in a greenhouse (McClain et al., 2006), but seedlings are rare in the field (Forseth and Innis, 2004). When seedlings do occur, survivorship is low (Abramovitz, 1983). The absence of seedlings is not due to freezing mortality of seeds. Seeds exposed to $-36^\circ$C germinated just as well as seeds exposed to $-10^\circ$C (Fig. 2.7). It is also not due to aborted fruit development in cold northern climates, since populations in New York and Leamington, Ontario set viable seed (Fig. 2.7, Robert Nurse, personal communication). No seedlings were observed at the Leamington population (Robert Nurse, personal communication). Instead, there appears to be another barrier to seedling recruitment in kudzu, making dispersal by seed an unlikely mechanism for kudzu range expansion.
A more likely mechanism of dispersal is the human-mediated transport of newly rooted stems or young plants. Within a population, kudzu vines can root at every node, resulting in as many as 6000 crowns per hectare (Bailey, 1939). Young crowns are common at the edges of kudzu stands, where they are readily dislodged and transported by roadside mowers or farm and construction equipment (H.A. Coiner, personal observation) As with other invasive species, kudzu occurs frequently in disturbed and high-traffic areas: of 78 kudzu populations in Illinois, 65 were along roadsides, near strip mines, and other sites of human disturbance (Bradley et al., 2010; Hodkinson and Thompson, 1997; Mack, 2003; McClain et al., 2006). Many of these sites may have been planted (McClain et al., 2006), or they may be the result of accidental transport by human vehicles. Either way, the vectors are human.

There is precedent for the human-mediated dispersal of kudzu. At the height of its popularity, kudzu was widely planted in the southeastern U.S. by the U.S. Soil Conservation Service and the U.S. Department of Agriculture (Bailey, 1939; Alderman, 2004). The primary motivation for this practice was the conservation of soils denuded by decades of severe erosion (Bailey, 1939; Winberry and Jones, 1973). The northeast did not have this legacy, so kudzu was not widely planted there. Thus, kudzu’s apparent rapid spread in the southeast may have been largely the result of this historical practice of assisted dispersal combined with its ability to expeditiously cover ground once established (Wilson et al., 2009). Now that kudzu is no longer being planted on large government-assisted scales, dispersal may have slowed, but it has not stopped; we show that kudzu has clearly moved northward in the last four decades (Fig. 2.1). We suggest that these new populations were probably established with the intentional or unintentional help of humans. If so, then kudzu already has the potential to expand northward, and the projections in Fig. 2.2 become under-estimates of kudzu’s potential range.

A second hypothesis that could explain kudzu’s northern range limit is that the correlation with the −20 to −22°C isocline is indeed robust, but that it represents a correlate
to a climatic limitation other than cold winters. In North America, cold winters can be associated with chilling temperatures (0–10°C) in the spring, suboptimal temperatures (< 20°C) in the summer, and more frequent extreme cold events (e.g., frosts) in spring and fall (Bryson and Hare, 1974; Martyn, 1992). Cool temperature episodes throughout the growing season can reduce carbon gain in warm-climate plants like kudzu by restricting stomatal opening, disrupting electron transport, slowing carbohydrate metabolism and impairing Rubisco function (Berry and Bjorkman, 1980; Sage and Sharkey, 1987; Allen and Ort, 2001). Simulations show that diurnal carbon losses due to reversible midday photoinhibition (due to high light alone) range from 6–9% of total carbon gain (Long et al., 1994; Werner et al., 2001). The additional stress of chilling temperatures in combination with high light should further reduce carbon gain (Bertamini et al., 2006; Takahashi and Murata, 2008). For example, the efficiency with which *Brassica rapa* converts incident light into dry matter drops by 33% when grown in chilling vs. non-chilling conditions (Farage and Long, 1991). Reductions in carbon gain could restrict kudzu’s competitive ability during the growing season, or cause mortality if carbon stores are insufficient to survive winter (Chapin et al., 1990; Mooney and Gartner, 1991; Mendham et al., 1981). Unfavorable carbon balance has, for example, been shown to increase black spruce mortality at its southern range limit (Way and Sage, 2008a). The remainder of this thesis will evaluate whether cool temperatures during the growing season cause reductions in kudzu growth and photosynthesis.

### 2.5.2 Implications for species distribution modeling and conservation

The two alternative hypotheses discussed above imply that kudzu’s future distribution is either correctly estimated or underestimated (Fig. 2.2). There is little evidence to support the alternative possibility that the projections over-estimate kudzu’s potential
range. Despite the absence of the most obvious mechanism (winter cold tolerance) behind the correlation in Fig. 2.1, the projections could still correctly identify a climatic constraint, even if additional experiments fail to identify a physiological mechanism. This is because it is impossible (or nearly so) to empirically test all direct and indirect effects of temperature on an organism in its native or naturalized community (Holt and Keitt, 2005; Guisan and Thuiller, 2005; Gaston, 2009; Colwell and Rangel, 2009). The main strength of using correlations to look at species distributions is that they account for all of the factors that control range limits, including history and stochastic factors that are difficult to test empirically (Kearney and Porter, 2009). Nevertheless, one non-climatic alternative hypothesis—that dispersal is limiting—is also compelling. If this hypothesis is true, then the projections in Fig. 2.2 are underestimates, and kudzu could have the potential—right now—to survive north of its current limit.

In the context of climate change, it is imperative that assumptions (like kudzu’s sensitivity to cold) be challenged in the lab. This is especially true for invasive species, for whom regulatory policy must be established. Many land managers and regulators have assumed kudzu cannot tolerate cold, which has led to this species being unregulated in Canada and in several northern U.S. states. Of the U.S. states where kudzu is already present at low densities (Fig. 2.1), Delaware, Michigan, New York and Indiana do not list kudzu as a noxious weed, which means that trade and transport of kudzu are legal in those states (U.S. Department of Agriculture, 2010). In Canada, kudzu has been recommended but not approved for regulation, so it can be commercially distributed and governments have no legal means to remove populations on private lands (Canadian Food Inspection Agency, 2010; Government of Canada, 2011). We show that evaluating the physiological basis of an assumed climatic restriction can help guide the allocation of financial resources to early detection and eradication programs for potentially noxious species. Doing so will minimize the cost of confronting invasions, even with the added
uncertainty of climate change. In the case of kudzu, there is no support for the hypothesis that cold winters exclude it from northern U.S. states and adjacent Canada, making this species a good candidate for regulation.
Chapter 3

Springtime chilling of kudzu
(*Pueraria montana* var. *lobata*) and its significance for future range expansion

This chapter is the product of a collaboration with Rowan F. Sage, who contributed ideas and helped with the writing.

3.1 Abstract

Kudzu is an Asiatic vine that is invasive in North America. Kudzu’s northern range limit is correlated with minimum winter temperature, but winter freezing mortality does not explain the correlation. This study evaluates whether chilling temperatures in the spring can explain kudzu’s northern range limit. Growth and net CO$_2$ assimilation rates ($A_{net}$) were measured for *ca.* 30 days after shoots emerged from kudzu plants growing naturally in Leamington, Ontario. In 2010, early-emerging shoots experienced an unusually severe
Chill (minimum air temperature ($T_{\text{min}}$)= 0.8°C); in 2011, shoots experienced a moderate chill ($T_{\text{min}}$= 5.6°C). In both years, shoot emergence was associated with a 10 days rolling mean temperature of ≥ 13°C. In 2010, the canopy had closed 20 days after the chill; in 2011, this took 17 days. Of the 50% of leaves that survived the 2010 chill, high $A_{\text{net}}$ was achieved in less than 10 days following the chill. Recovery was complete in 3 days in 2011. Of the 85% of stems that survived the chill in 2010, growth recovered immediately. In 2011 there was no mortality and no growth delays. Stem elongation rate was best explained by maximum air temperature ($T_{\text{max}}$). Growth rates were greater than 10 cm d$^{-1}$ when $T_{\text{max}}$ ≥ 25°C and less than 5 cm d$^{-1}$ when $T_{\text{max}}$ < 15°C. $A_{\text{net}}$ was best explained by $T_{\text{min}}$ in both years; maximum rates (> 15 μmol m$^{-2}$ s$^{-1}$) required minimum morning temperatures of at least 12.5°C. At $T_{\text{min}}$ < 10°C, $A_{\text{net}}$ was less than 5 μmol m$^{-2}$ s$^{-1}$.

The probability of chill events and favorable temperatures after budbreak was calculated for Leamington, and for three more northern locations in Ontario where kudzu is not present. The results do not support the hypothesis that chilling temperatures prevent kudzu success at its current range limit. Several hundred km north of its current range limit, freezing temperatures or sequential chills may reduce kudzu’s viability, but only if kudzu emerges early due to a warm spring. If kudzu avoids freezing by emerging later, temperatures are as favorable for early season growth and photosynthesis in the three northern locations as they are in Leamington. There is no strong evidence that spring chilling is limiting kudzu migration into other parts of Ontario, so it should be made a priority for invasive species management.

3.2 Introduction

Kudzu (Pueraria montana var. lobata (Willd.) Ohwi) is an invasive vine originating in East Asia that is expanding its naturalized range northward in North America (Chapter 2; Ziska et al., 2011; McClain et al., 2002). Kudzu grows rapidly and can exploit a wide
range of soil conditions, which made it popular for erosion control and soil conservation in the early 20th century (Bailey, 1939; Winberry and Jones, 1973). Kudzu is now considered a noxious weed for its ability to reduce biodiversity, degrade local air quality through emissions of isoprene and nitrous oxides, and cover cars, buildings, and natural vegetation with a tangle of vines (Sharkey and Loreto, 1993; Forseth and Innis, 2004; Hickman et al., 2010; Blaustein, 2001). Kudzu is a thermophilic species that is thought to be intolerant of cold temperatures (Miller and Edwards, 1983; Shurtleff and Aoyagi, 1985; Winberry and Jones, 1973). Nevertheless, kudzu has established successful populations in temperate and continental areas such as upstate New York and southern Canada, suggesting that it may be more cold tolerant than previously suggested (Chapter 2; Ziska et al., 2011; McClain et al., 2006; Frankel, 1989).

Kudzu’s northern range limit coincides historically and currently with the −20°C minimum winter temperature isocline, but the mechanism behind this relationship is unknown (Chapter 2; Sasek and Strain, 1990). Previous work has shown that the obvious mechanism—winter mortality—does not explain the correlation because many of the overwintering tissues are protected underground (Chapter 2). If kudzu is a thermophilic species, however, then other low temperature correlates to minimum winter temperature—such as short frost-free periods, greater annual temperature variability (continentality), and high probability of cold events in the growing season—could prevent kudzu establishment (Martyn, 1992). Here, we examine the role of spring chilling in establishing kudzu’s northern range limit.

To affect kudzu’s northern range limit, the physiological effects of chilling must disrupt its life history (Larcher, 2003). Kudzu is a semi-woody perennial vine that forms overwintering buds that produce green shoots in the spring (Tsugawa et al., 1988). Kudzu invasiveness depends on vigorous spring growth to effectively suppress competitors and capture available sunlight (Bailey, 1939). The completion of the annual life cycle of most temperate deciduous species requires the accumulation of sufficient carbohydrate reserves
over the summer to survive the winter and initiate bud break in the spring (Mooney and Gartner, 1991; Chapin et al., 1990). Summers are short at high latitudes, so if buds break too late because of chilling temperatures, then kudzu may not have time to develop a canopy and assimilate enough carbon to ensure winter survival or grow fast enough in the spring. Similarly, if young shoots are killed by a late spring cold event, kudzu may lose its competitive advantage if co-occurring species recover more quickly. If shoots are not killed, growth may still be too slow in cool temperatures to compete with other species.

The physiological effects of spring chill events restrict the range limits of many warm-adapted crop and ornamental species, such as cotton, mango, tomato, and corn (Lyons et al., 1979; Berry and Raison, 1981; Allen and Ort, 2001). Chilling temperatures reduce both the activity and quantity of enzymes involved in photosynthetic carbon metabolism, including Rubisco and fructose-1,6-bisphosphatase, in chilling-sensitive vs. chilling-resistant species (Allen and Ort, 2001; Naidu et al., 2003; Bertamini et al., 2007; van Heerden et al., 2003). Chilled leaves with slowed carbon metabolism that are exposed to high light can experience damage to the photosynthetic electron transport chain. Such conditions can cause pigment bleaching and damage to D1, a core protein in photosystem II (Huner et al., 1998; Long et al., 1994; Powles et al., 1983), resulting in reduced photosynthetic efficiency. Chilling-induced reductions in photosynthesis are not always the result of damage, however. Riesling grapevines, which are cultivated in Germany and southern Canada where chilling is common, experience reduced photosynthesis following low night temperature treatments as a result of dynamic regulatory processes rather than damage (Bertamini et al., 2006). Plants that employ such protective processes are able to recover quickly from chilling conditions.

In chilling-sensitive species, reductions in carbon metabolism due to chilling can reduce carbon gain and growth. For example, mild chilling can reduce potential carbon gain by up to 8.5% in sunlit leaves of *Quercus coccifera*, a mediterranean oak (Werner et al., 2001). Grapevines growing in cool vs. warm microsites that differed in temperature by
only 1–3 °C had slower growth rates and up to 20% less carbon gain (Hendrickson et al., 2004a). Chilling can also result in persistent reductions in growth rates. Chilled cotton seedlings had lower relative growth rates in a warm recovery period, due to stomatal and non-stomatal effects on photosynthesis (DeRidder and Crafts-Brandner, 2008). Stems originating from grapevine buds chilled to ~12 °C grew at about 60% the rate of stems originating from unchilled buds several weeks after treatments ceased (Keller, 2010). If chilling temperatures are restricting kudzu range expansion at its northern range limit, then there should be evidence that chilling temperatures result in persistent reductions in growth and photosynthesis. In this study, my objective is to evaluate the hypothesis that chilling conditions cause persistent reductions in kudzu growth and photosynthesis. To test this hypothesis, I characterized the response of kudzu growth and photosynthesis to springtime chilling in a northern population.

3.3 Methods and Materials

3.3.1 Field site

All field experiments were performed on naturalized plants growing at the first documented Canadian kudzu site in Leamington, Ontario (42.02°N, 82.74°W). The Leamington site is ca. 120 × 50 m in size and 7 to 40 years old. The site covers a steep south-facing embankment on the north shore of Lake Erie. At the top of the embankment, the site borders a cultivated field; Lake Erie is at the bottom (Fig 1.2 B).

3.3.2 Environmental conditions

Daily air temperatures were monitored at the Environment Canada weather station in Harrow, Ontario, Canada, ca. 20 km west of the kudzu site (Harrow CDA, 42.03°N, 82.90°W, elevation = 191 m.a.s.l., Climate ID = 6133362) (www.climate.weatheroffice.gc.ca). These weather conditions were used to determine the timing of measurements. Leaf tem-
peratures were measured with 36-gauge thermocouples (Type T, Omega, Laval, QC) connected to a datalogger (VL-1700, Veriteq Instruments, Richmond, BC). Thermocouples were attached to the underside of leaves with porous paper medical tape.

### 3.3.3 Growth measurements

Stem elongation was measured on shoots located along two or three transects that ran east to west through the patch. On the first day, 12 to 20 shoots along each transect were marked with a loose cable tie below the sixth node. The same shoots were followed throughout the study. The elongating tip usually contains 3–4 immature nodes; the first expanding leaf is usually at the fourth node (Tsugawa et al., 1987). Shoot length was measured from the marked node to the shoot tip with a seamstress tape. Growth rate for each shoot was calculated as the difference in length between sequential measurements, divided by the difference in time, which was calculated in decimal days. There were no differences between the transects, so the calculated growth rates were pooled to produce a mean growth rate per day. Stem diameter between the third and fourth node from the base of the stem was measured on the last day of the study in 2011. Damage to stems due to chilling was assessed visually on May 9–10, 2010 (Jul = 129–130). Moderately damaged shoots had leaves that were curling, moderately necrotic, or dessicated. Severely damaged shoots had leaves that were severely dessicated or necrotic, and shoot tips that were wilted, water-soaked, or shriveled.

### 3.3.4 Leaf gas exchange and chlorophyll fluorescence

Simultaneous leaf gas exchange and chlorophyll fluorescence measurements were determined on leaves located on top of the embankment. In 2011, chlorophyll fluorescence was not measured. On the first day of each experiment, 8–12 leaves on separate shoots were marked with a loose cable tie. The most mature leaves available were chosen, but they were not yet fully expanded; length measurements along the midvein of the centre leaflet
showed that they expanded from 55 ± 2.4 mm on May 4 (Jul = 124) to 71 ± 3.3 mm on May 9, 2010 (Jul = 129). Leaves were also chosen to have similar net CO\textsubscript{2} assimilation rates (\(A_{\text{net}}\)) on the first day of the study. Gas exchange was measured on the same set of leaves before and after chill events. Recovery from the chill was measured two or three times during the subsequent 20 days. Ten to eleven days after the chill event in both years, the original set of leaves was compared to a second set of leaves that had expanded after the chill. In 2010, a third set of leaves was assessed 20 days after a chill event.

\(A_{\text{net}}\) was measured using an open-type portable gas exchange system with an integrated fluorescence head (LI–6400 and 6400–40, LI–COR, Lincoln, Nebraska, U.S.A.). Conditions in the cuvette were saturating light (1100–1400 \(\mu\text{mol}^{-1} \text{m}^{-2} \text{s}^{-1}\)) and an ambient CO\textsubscript{2} concentration of 390 \(\mu\text{mol}^{-1}\) mol. Leaf-air vapor pressure deficit (VPD) was maintained between 1–1.5 kPa. Leaf temperature was maintained between 23–30 °C except on the first day of the cold event, when it was 18 °C in 2010 and 21 °C in 2011 (Table C.1). Predawn \(F_v/F_m\) was measured on the study leaves in 2010. Ten days after the chill in 2010, predawn \(F_v/F_m\) was also measured on 15–16 leaves along the same three transects used for growth measurements. A warm-temperature control value for \(A_{\text{net}}\) was measured on six fully expanded, outer canopy leaves using the above cuvette conditions on July 20, 2011 (Jul = 201).

To check for irreversible damage to photosystem II (PSII), fluorescence yield was measured on six mature leaves acclimated to full sunlight (ca. 1900 \(\mu\text{mol}^{-1} \text{m}^{-2} \text{s}^{-1}\)) before, during, and after the chill event in 2010. Following yield measurements, leaves were harvested, sealed in clear plastic bags with a moist paper towel, and allowed to recover for 30 min at 0 \(\mu\text{mol}^{-1} \text{m}^{-2} \text{s}^{-1}\). Fluorescence yield was then remeasured and fluorescence parameters were calculated as described in Kornyeyev and Holaday (2008).

To evaluate whether \(A_{\text{net}}\) is related to morning chilling, pooled \(A_{\text{net}}\) values from both years were plotted vs. measurement temperature and minimum temperature the night before, which typically occurs just before sunrise. The youngest leaves (Day 1 in
both years) and the leaves that appeared to have sustained moderate to severe damage from chilling were excluded (Fig. 3.2). Minimum morning temperature ($T_{\text{min}}$) on the day of measurement was parameterized as a discrete variable with levels: $T_{\text{min}} < 10^\circ C$, $T_{\text{min}} = 10–12.5^\circ C$, and $T_{\text{min}} > 12.5^\circ C$.

### 3.3.5 Climate analysis

To determine whether springtime chilling temperatures may limit kudzu’s northward range expansion in Ontario, the climate in Leamington was compared to the climate in Guelph (277 km NE from Leamington), Barrie (410 km NE), and North Bay (659 km NE). Daily weather data was obtained from Environment Canada weather stations for the 10 y period 2002–2011 (www.climate.weatheroffice.gc.ca). The stations were Harrow CDA (Section 3.3.2), Fergus MOE (~ 20 km NW of Guelph, 43.70°N, 80.38°W, elevation = 396 m.a.s.l., Climate ID = 6142402), Barrie-Oro (for 2004–2011 only, 44.48°N, 79.55°W, 289 m.a.s.l., Climate ID = 6117700), Barrie WPCC (for 2002–2003 only, 44.38°N, 79.69°W, 221 m.a.s.l., Climate ID = 6110557), and North Bay A (46.36°N, 79.42°W, 370 m.a.s.l., Climate ID = 6085700).

Since temperature events are only relevant after kudzu has emerged, the date of budbreak was estimated at each location. The first day of the study was taken to represent the day of budbreak because the day of first emergence was not observed. The mean temperature conditions in the 10 days prior to the first day of measurements were 13–14°C in both years, so 13°C was taken to be the temperature threshold for budbreak (Table 3.1). To estimate the date of budbreak, the 10 days rolling average of mean daily temperature was calculated for each year, and the first day in May or June where this average was $\geq 13^\circ C$ was taken as the day of budbreak (Table 3.1). May 1 was the earliest allowed date of budbreak because mean temperatures were $\geq 13^\circ C$ in April 2010 in Leamington, but kudzu did not emerge until that threshold was met again in early May. The dates were then averaged to estimate a mean date of budbreak for each
Table 3.1 – Air temperatures during the study at Harrow CDA meteorological station in Harrow, Ontario (~20 km west of Leamington, 42.03°N, 82.9°W, elevation = 191 m.a.s.l., Climate ID = 6133362, WMO ID = 71298, TC ID = XHA) (www.climate.weatheroffice.gc.ca). Dates and temperatures during each 2 day chill event are shown, along with temperatures before and after the chill event. Mean air temperature is shown with mean minimum and mean maximum temperatures in parentheses. Absolute minimum and maximum temperatures recorded during the period are in square brackets.

<table>
<thead>
<tr>
<th>Year</th>
<th>10 d prior to study, °C</th>
<th>Chill event, °C</th>
<th>1–10 d after chill, °C</th>
<th>11–20 d after chill, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td><strong>Apr 23–May 3</strong></td>
<td><strong>May 9–10</strong></td>
<td><strong>May 11–20</strong></td>
<td><strong>May 21–30</strong></td>
</tr>
<tr>
<td></td>
<td>13.4 (8.6, 18.2)</td>
<td>6.65 (0.9, 12.4)</td>
<td>12.8 (8.3, 17.3)</td>
<td>21.0 (15.4, 26.6)</td>
</tr>
<tr>
<td></td>
<td>[0.8, 26.3]</td>
<td>[0.8, 12.9]</td>
<td>[5.2, 25.1]</td>
<td>[13.0, 29.3]</td>
</tr>
<tr>
<td>2011</td>
<td><strong>May 4–13</strong></td>
<td><strong>May 15–17</strong></td>
<td><strong>May 18–27</strong></td>
<td><strong>May 28–Jun 7</strong></td>
</tr>
<tr>
<td></td>
<td>14.0 (8.5, 19.5)</td>
<td>9.0 (6.6, 11.3)</td>
<td>16.4 (11.8, 21.0)</td>
<td>18.4 (13.5, 23.2)</td>
</tr>
<tr>
<td></td>
<td>[0.4, 25.6]</td>
<td>[5.6, 12.7]</td>
<td>[8.3, 26.7]</td>
<td>[8.3, 31.2]</td>
</tr>
</tbody>
</table>
The climates at the four locations were analyzed using the 30 day period following estimated dates of budbreak for the 10 year period 2002–2011. This represents the time required for kudzu to develop ∼ 75% of its canopy (Tsugawa et al., 1987). To account for some of the uncertainty in the budbreak estimate, probabilities were also calculated for the earliest and latest observed budbreaks in the 10 year period. The probability of a damaging chill event in a given year was estimated by counting the number of years (out of ten) where $T_{\text{min}}$ met the criteria on at least one day. The criteria were based on results from this chapter and Chapter 2) and were: $T_{\text{min}} = 0–2\, ^\circ\text{C}$, which would have effects similar to chills reported here; $T_{\text{min}} = -2–0\, ^\circ\text{C}$, which would presumably have more severe effects, and $T_{\text{min}} < -2\, ^\circ\text{C}$, which could be lethal (Fig. B.2D). The intensity of low temperature events were estimated by counting the number of years where more than one consecutive day met the criteria. When days that met the criteria were separated by one day, that day was assumed to be a part of the cold event. The probability of separate sequential chill events in a given year was estimated by counting the number of years (out of ten) where the criteria were met on days that were separated by at least four days of warmer temperatures.

To estimate whether kudzu would be able to achieve high rates of photosynthesis and growth at other locations in Ontario, the probability of temperatures falling above or below a physiological temperature threshold on a given day were calculated. The thresholds, which were inferred from Figs. 3.5 and 3.6, were: $T_{\text{max}} < 15\, ^\circ\text{C}$, which slows growth rates to less than 5 cm d$^{-1}$; $T_{\text{max}} \geq 25\, ^\circ\text{C}$, above which growth rates are greater than 10 cm d$^{-1}$; $T_{\text{min}} < 10\, ^\circ\text{C}$, which slows $A_{\text{net}}$ to less than 5 $\mu$mol$^{-1}$ m$^{-2}$ s$^{-1}$; and $T_{\text{max}} \geq 12.5\, ^\circ\text{C}$, which is needed for $A_{\text{net}}$ to be greater than 10 $\mu$mol$^{-1}$ m$^{-2}$ s$^{-1}$. Probabilities were estimated by adding up the number of days that met the criteria divided by the total number of days. R Project software v. 2.12.1 was used for all analyses (R Development Core Team, 2011).
3.4 Results

3.4.1 Environmental conditions

In 2010, the study began on May 4 (Julian Day (Jul) = 124), ten days earlier than it began in 2011. Air temperatures in the ten day period prior to the beginning of both measurement periods were similar, averaging 13–14°C (Table 3.1, Fig. C.2). In 2010 a severe chill occurred on May 9–10 (Jul = 129–130); the days were clear and air temperatures dropped to 0.8°C. In 2011, a chill on May 15–17 (Jul = 135–137) was milder; all days were overcast, and temperatures did not go below 5.6°C (Table 3.1, Fig. C.2). Temperatures were also cooler (mean = 12.8°C) in the ten day period following the chill event in 2010 than they were in 2011 (mean = 16.4°C) (Table 3.1, Fig. C.2). In 2011, a second more mild two day cold front (minimum = 8.3°C) arrived nine days (Jul = 146) following the first chill event in the study (Fig. C.2). In the subsequent ten day period (May 21–30, Jul = 141–150), temperatures continued to warm in 2010 (mean = 21.0°C) while they were marginally cooler in 2011 (May 28–Jun 7, Jul = 148–158, mean = 18.4°C) (Table 3.1). In 2011, leaf temperature was logged during the transition into the chill event (Table C.2). Minimum leaf temperatures stayed within ±3°C of air temperature (Table C.2). In contrast, maximum leaf temperatures were as much as 12°C warmer than air temperature prior to the chill, dropping to within ±4°C of air temperature in the overcast days during and after the chill (Table C.2).

3.4.2 Qualitative description of chill impacts

On the first day of the study in both years, kudzu shoots had just begun to emerge. Shoots emerged approximately 10 days later in 2011 than in 2010. In 2010, the longest shoots were eight nodes long (< 50 cm), while in 2011, the longest shoots were six nodes long (< 10 cm) (data not shown). Elongating shoots were scarce, and the grey stems of dead and overwintering kudzu vines visually dominated the site (Fig. 3.1 A, D). Other
Figure 3.1 – Photographs of the Leamington kudzu population in 2010 (A–C) and 2011 (D–F). On the first day of the study in both years (A, D), the site was dominated by species other than kudzu. Note the ten day lag in 2011 relative to 2010, due presumably to the cooler spring of 2011. Dead and overwintered stems give the slope a grey colour. Ten days after the chill in 2010 (B), the site still appears grey, but after a similar length of time in 2011 (E), emerging shoots make the slope noticeably greener. After six more days in 2011 (F), and ten more days in 2010 (C), the kudzu canopy is nearly closed.
species present included *Alliaria petiolata*, an unidentified grass, an *Equisetum* spp., and an unidentified mint (Fig. 3.1 A, D). The phenology of these species and the surrounding trees also appeared to be delayed by the cold spring in 2011 (Fig. 3.1 A, D). Ten days after the chill in 2010 (Fig. 3.1 B), the site still appears grey, but in 2011 (Fig. 3.1 E), it is noticeably greener due to kudzu shoots emerging after a similar length of time. At this time in both years, the kudzu canopy has still not closed, and other species are evident. However, after six more days in 2011 (June 3, Fig. 3.1 F), and ten more days in 2010 (May 30, Fig. 3.1 C), the canopy had closed.

![Figure 3.2](image-url) – Photographs of representative leaves in 2010 (A–D) and 2011 (E–G). The same gas exchange leaves (A, D, E, F) are shown pre-chill (A, E) and 10 days post-chill (B, F). Note the necrosis and crinkling in the 2010 leaf (B) compared to the 2011 leaf (F). Newly formed leaves used for gas exchange on May 20, 2010 (C, D) show the large variation in leaf quality observed that year. The second cohort of leaves measured on May 28, 2011 were more uniformly healthy (G).

In 2010 and in 2011, there were two cohorts of leaves used for gas exchange. The first cohort expanded prior to (and during) the chill (Fig. 3.2 A–B, E–F), while the sec-
ond cohort was assumed to have experienced the chill as buds or immature leaves, and expanded after the chill (Fig. 3.2 C–D, G). In 2010 there was a third cohort measured 20 days after the May 9–10 chill (not shown). On the first day of the study in both years, leaves were small but otherwise appeared healthy (Fig. 3.2 A, E). Following the chill event in 2010, seven of the twelve leaves used for gas exchange had broken off at the petiole (not shown), suggesting that they had died in the chill. The five remaining leaves exhibited moderate to extensive mechanical damage, necrosis, and curling due presumably to uneven cell expansion (Fig. 3.2 B). In some of the expanded leaves in this cohort, chlorophyll development appeared to be unimpeded by the chill (Fig. 3.2 B), as evidenced by the dark green colour of the leaves, while others showed symptoms of chlorosis (similar to the leaf in Fig. 3.2 D). These symptoms were absent or less pronounced in leaves exposed to mild chilling in 2011 (Fig. 3.2 F).

The second cohort of leaves measured on May 20, 2010 (Jul = 140, 10 days following the chill) were selected to demonstrate the variety of chill effects on a cohort of leaves that were immature during the chill (Fig. 3.2 C, D). Leaves were much less variable in

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**Figure 3.3 (following page)** – Growth and photosynthesis during the May 2010 chill event. (A) Mean (solid line), minimum and maximum (dashed lines) daily air temperature at Harrow meteorological station. (B) Mean (± se) stem elongation rate of stems that appeared healthy (circles, \( N = 20 \)), damaged (upright triangles, \( N = 32 \)), or dead (inverted triangles, \( N = 9 \)) following the chill event. Values are the mean growth rate since the preceding measurement. Solid diamonds are the maximum elongation rate observed on a given day. (C) Mean (± se) \( A_{\text{net}} \), (D) stomatal conductance, and (E) \( C_l/C_a \) measured repeatedly on 5–12 marked leaves (circles) at the top of the slope. Solid diamonds in (C) are maximum \( A_{\text{net}} \) measured on that day. Triangles in (C)–(E) represent mean (± se) \( A_{\text{net}} \) of new leaves that had expanded after the chill that appeared to be healthy (upright triangles, \( N = 5–10 \)) or damaged (inverted triangles, \( N = 6 \)) by the chill. There were no damaged leaves on Jul = 150.
Chapter 3. Springtime chilling

Air temperature, °C

Stem elongation rate, cm d⁻¹

Net CO₂ assimilation rate, µmol CO₂ m⁻² s⁻¹

Stomatal conductance, mol H₂O m⁻² s⁻¹

Ci/Ca

Julian day in 2010
2011, so the comparison was not made (Fig. 3.2G). In 2010, some leaves appeared to have developed normally (Fig. 3.2C) while others exhibited moderate to severe signs of developmental abnormalities such as uniform or localized chlorosis, necrotic regions, and curling or stunting (Fig. 3.2D). Co-occurring species showed similar abnormalities following the 2010 chill (data not shown). The third cohort of leaves (measured on May 30, 2010 (Jul = 150, 20 days following the chill), were large and appeared healthy.

3.4.3 Stem elongation rate

Stem elongation was measured on 9–19 stems along three transects in 2010. In 2011, 16–17 stems were measured along two transects. Growth rates from all transects were pooled. In 2010, most (91%) of these early emerging stems originated from unrooted stems buried in the leaf litter while the remainder grew from crowns (data not shown). In both years, stem elongation rates appeared to track changes in air temperature (Figs. 3.3 A–B, 3.4 A–B). In 2010, the mean growth rate of all stems was $4.5 \pm 0.4$ and $5.5 \pm 0.3 \text{ cm d}^{-1}$ on the two days prior to the chill (May 5–6, Jul = 125–126). On the two chill days, mean growth rates dropped to $2.0 \pm 0.2 \text{ cm d}^{-1}$ (maximum = 5.0 cm d$^{-1}$) (Fig. 3.3 B). Of the 61 stems followed through the chill, nine appeared severely damaged by the chill, 32 were

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**Figure 3.4 (following page)** – Growth and photosynthesis during the 2011 chill event. (A) Mean (solid line), minimum and maximum (dashed lines) daily air temperature at Harrow meteorological station. (B) Mean ($\pm$ se) stem elongation rate, measured repeatedly on 34 marked stems. Solid diamonds are the maximum elongation rate observed on a given day. Rates represent the average growth since the preceding measurement. (C) Mean ($\pm$se) $A_{\text{net}}$, (D) stomatal conductance, and (E) $C_i/C_a$ measured repeatedly on 5–12 marked leaves (circles) at the top of the slope. Solid diamonds in (C) are the maximum $A_{\text{net}}$ measured on that day. Triangles in (C)–(E) represent means ($\pm$ se) of a cohort ($N = 6$) of healthy-looking leaves.
Chapter 3. Springtime chilling

A. Air temperature, °C

B. Stem elongation rate, cm d⁻¹

C. Net CO₂ assimilation rate, µmol CO₂ m⁻² s⁻¹

D. Stomatal conductance, mol H₂O m⁻² s⁻¹

E. Cᵢ/Cₐ

Julian day in 2011
moderately damaged, and 20 appeared healthy. The growth rate of moderately damaged stems was similar to that of healthy stems for the remainder of the study, while the growth rate of severely damaged stems was reduced to zero following the chill and did not recover (Fig. 3.3B). After ten days, growth rates were still lower than those prior to the chill, but temperatures during that period were also still cool (mean = 12.8°C, Table 3.1). Twenty days after the chill, when mean temperatures had increased to about 21.0°C, growth rates were nearly double pre-chill values (mean = 8.3 ± 0.3 cm d⁻¹, max = 11.5 cm d⁻¹, Fig. 3.3 A–B).

In 2011, the chill event was less severe, and temperatures were warmer after the chill (Table 3.1). Stem growth attained higher rates more quickly than in 2010 (Fig. 3.4 A–B). Prior to the chill, elongation rates were 3.7 ± 0.3 cm d⁻¹ (Fig. 3.4 A–B). The maximum growth rate observed before the chill (6.8 cm d⁻¹) was about 2/3 that observed in 2010 (9.4 cm d⁻¹, Fig. 3.3 B), but the shoots were also younger. As with the severe chill in 2010, the moderate chill in 2011 slowed growth (to 1.6 ± 0.9 cm d⁻¹) (Fig. 3.4 B). One day later, when temperatures had begun to warm, growth was still low at 1.4 ± 0.1 cm d⁻¹. Growth rates recovered to pre-chill levels over the next three days (3.8 ± 0.4 cm d⁻¹) (Fig. 3.4 B). By the end of May, growth rates were 8.5–8.6 cm d⁻¹ in both years (Fig. 3.3 B, Fig. 3.4 B).

To evaluate whether growth rates are best predicted by minimum (\(T_{\text{min}}\)) or maximum (\(T_{\text{max}}\)) daily temperatures, pooled growth measurements for both years of field experiments were fit with eleven separate temperature variables describing conditions during the time period (1–10 days) between measurements (Table C.3). Stems in 2010 that were severely damaged by the chill (inverted triangles in Fig. 3.3 B) were excluded. The best temperature predictor was the average \(T_{\text{max}}\) during the period (ANOVA: \(F_{1,415} = 309.7, P(\text{<}|F|)<0.001\), adj. \(R^2 = 0.43\), AIC = 1790, Table C.3). The variance in rate increases with increasing temperature, so separate models were fit to the upper and lower quintiles (20%) of the data as well as to the mean (Fig. 3.5). The \(x\)-intercepts of the lines fit to
the maximum and minimum quintiles are 5.7°C and 9.5°C, respectively; mean growth is zero at 6.4°C (Fig. 3.5).

The fastest growing stems are the most responsive to temperature; mean $T_{\text{max}}$ explains 73% of the variance in the fastest 20% of stems, but only 40% in the slowest 20%. The fastest stems are also the thickest (Fig. C.3). Stems with a diameter of 5 mm grew 4 times faster than stems half that diameter (Fig. C.3). Maximum growth rates were 10 cm d$^{-1}$ or higher when mean $T_{\text{max}}$ exceeded 25°C. At 15°C and below, growth did not exceed 4.8 cm d$^{-1}$ (Fig. 3.5).

To test whether growth was depressed in the days following the chill event, the expected growth rate was calculated using equations developed using a similar independent dataset collected during mid-summer in July 2011 in Leamington (Chapter 4, Fig. 4.1). Observed mean growth rates were no different from what would have been expected (given the cool maximum daily temperatures) had there been no chill (paired Student’s $t$-test: $t_{\nu=4} = 0.178$, $P = 0.87$).

### 3.4.4 Leaf gas exchange

Five to twelve leaves were measured on each day of leaf gas exchange assessments in 2010 and 2011. Measurement temperature was 24–31°C in 2010 and 23–27°C in 2011, except on chill days, when it was 19°C in 2010 and 21°C in 2011 (Table C.1). As with growth rate, $A_{\text{net}}$ appeared to mirror changes in air temperature (Figs. 3.3 A–C, 3.4 A–C). In 2010, mean $A_{\text{net}}$ prior to the chill was about half that in 2011 (3.9–4.7 vs. 7.6 $\mu$mol$^{-1}$ m$^{-2}$ s$^{-1}$, Figs. 3.3 C, 3.4 C). The severe 2 days chill in 2010 reduced $A_{\text{net}}$ by 70–80% (to 0.8–1.1 $\mu$mol$^{-1}$ m$^{-2}$ s$^{-1}$) relative to pre-chill values (Fig. 3.3 C). The more moderate 3 day chill in 2011 reduced $A_{\text{net}}$ by nearly 60% to 3.2 $\mu$mol$^{-1}$ m$^{-2}$ s$^{-1}$ (Fig. 3.4 C)—still triple the rates during the chill in 2010. Stomatal conductance declined to a similar degree as $A_{\text{net}}$ during the chilling events, causing calculated $C_i/C_a$ to change little during the chill (Figs. 3.3 C–E, 3.4 C–E).
Figure 3.5 – Stem elongation rate as a function of mean maximum temperature over the 1–10 day period pertaining to the measurement. Open symbols are the overall means, solid symbols are means of the fastest quintile (20%) and crossed symbols are the means of the slowest quintile. Circles represent growth rates on each day of measurement in Leamington, Ontario in 2010 and 2011 (Figs. 3.3, 3.4). Regression lines are fit to the Leamington data only. Regression lines are extrapolated to the x-axis to estimate the temperature were growth is zero. The regression for the upper and lower quintiles are $y = 0.51x - 2.9$ (adj. $R^2 = 0.73$) and $y = 0.19x - 1.8$ (adj. $R^2 = 0.40$), respectively. The regression fit to the overall mean was $y = 0.33x - 2.1$ (adj. $R^2 = 0.43$). The x-intercepts of the extrapolated lines predict zero growth will occur between 5.7°C and 9.5°C.
Chapter 3. Springtime chilling

Table 3.2 – Observed mean (maximum) growth rates 1–11 days after chill exposure compared with expected growth rates in the absence of the chill. Stems severely damaged by the chill in 2010 were excluded. Expected rates were calculated using daily maximum temperatures, and linear regressions fit to a dataset similar to that in Fig. 3.5 that was collected during a heat wave in July 2011 when there should not have been any lag effects due to chilling temperatures (Chapter 4, Fig. 4.1). Mean observed and expected rates are not significantly different (paired Student’s $t$-test: $t_4 = 0.178$, $P = 0.87$)

<table>
<thead>
<tr>
<th>Year</th>
<th>Jul</th>
<th>No. days after chill</th>
<th>Observed rate (cm d$^{-1}$)</th>
<th>Expected rate (cm d$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>140</td>
<td>10</td>
<td>3.5 (6.1)</td>
<td>3.2 (6.2)</td>
</tr>
<tr>
<td>2011</td>
<td>138</td>
<td>1</td>
<td>1.4 (2.8)</td>
<td>3 (6.2)</td>
</tr>
<tr>
<td></td>
<td>141</td>
<td>3</td>
<td>3.8 (6.7)</td>
<td>4.6 (5.4)</td>
</tr>
<tr>
<td></td>
<td>148</td>
<td>11</td>
<td>5.4 (11.8)</td>
<td>5.1 (9.0)</td>
</tr>
</tbody>
</table>
Recovery from the moderate chill in 2011 started on the first day of warmer temperatures. On May 18, 2011 (Jul = 138), an increase in $T_{\text{min}}$ to 11.2 °C was associated with a 44% increase in $A_{\text{net}}$ from the previous cold day (to 4.6 μmol$^{-1}$ m$^{-2}$ s$^{-1}$) (Fig. 3.4 C). On the fourth day following the chill (May 21, Jul = 141), $A_{\text{net}}$ was 6.6 μmol$^{-1}$ m$^{-2}$ s$^{-1}$, about 85% of pre-chill rates, but temperatures were also cooler than prior to the chill (Fig. 3.4 C). Leaves that had been recovering for 11 days from the moderate chill in 2011 (May 28, Jul = 148) had an $A_{\text{net}}$ of 14.9 μmol$^{-1}$ m$^{-2}$ s$^{-1}$, 70% higher than severely chilled leaves. Relative to $A_{\text{net}}$ measured prior to the chill, $A_{\text{net}}$ approximately doubled after 10 or 11 days of recovery in both years (Figs. 3.3 C, 3.4 C).

In 2010, leaves that expanded prior to the chill fared better than the cohort of leaves that had been exposed to the chill at early developmental stages (Figs. 3.2 C–D, 3.3 C). Young expanding leaves that appeared to have experienced moderate to severe developmental problems during the chill (e.g., Fig. 3.2 D) had very low assimilation rates ($2.0 \pm 0.6$ μmol$^{-1}$ m$^{-2}$ s$^{-1}$), while leaves that were visually less affected (e.g., Fig. 3.2 C) had assimilation rates that were more similar to the cohort that had expanded prior to the chill ($6.3 \pm 0.6$ vs. $8.7 \pm 2.2$ μmol$^{-1}$ m$^{-2}$ s$^{-1}$, Fig. 3.3 C). The leaf with the maximum rate measured on May 20, 2010 (Jul = 140) was one that had expanded prior to the chill (Fig. 3.3 C). These effects disappeared by the end of May, when a third cohort of leaves had rates similar to summer levels (Chapter 4, Fig. 3.3 C).

To evaluate whether $A_{\text{net}}$ is related to morning chilling, pooled $A_{\text{net}}$ values from both years were plotted vs. measurement temperature and minimum morning temperature (Fig. 3.6, Table C.4). Minimum morning temperature explained 49% of the variance in the data ($F_{2,88} = 115.4$, $P(< |F|) \leq 0.001$), but there was no interaction with measurement temperature (Table C.4). Morning temperatures below 10 °C depress $A_{\text{net}}$ below 5 μmol$^{-1}$ m$^{-2}$ s$^{-1}$ at measurement temperatures ranging from 17–28 °C. $A_{\text{net}}$ greater than 10 μmol$^{-1}$ m$^{-2}$ s$^{-1}$, which approaches levels measured in summer, was achieved on days when $T_{\text{min}}$ was at least 12.5 °C.
Figure 3.6 – Net CO₂ assimilation rate ($A_{\text{net}}$, mean ± se) as a function of measurement temperature when minimum morning temperature on the day of measurement was < 10°C (open circles), 10–12.5°C (crosses), or > 12.5°C (solid circles). Each point represents a single measurement on 8–13 leaves on each of 9 days in spring 2010 or 2011. The youngest leaves (Day 1 of each study) were excluded, as were leaves that were visibly damaged after the chill in 2010. $A_{\text{net}}$ of six representative upper canopy leaves was measured in Leamington on July 20, 2011 (triangle) as a warm temperature control. Loess (local) regression fits to summer $A_{\text{net}}$ vs. measurement temperature curves measured in Leamington in June and July 2011 (from Fig. 4.5 A) are shown as solid and dashed lines, respectively. Minimum morning temperature was significant ($F_{2,88} = 115.4$, $P(< |F|) \leq 0.001$), and there was no interaction with measurement temperature ($F_{2,88} = 1.4$, $P(< |F|) = 0.26$).
3.4.5 Chlorophyll fluorescence

Chlorophyll fluorescence was measured in 2010 but not in 2011 (Fig. 3.7). Mean predawn $F_v/F_m$ in the three days prior to the chill was 0.69, ranging from 0.55 to 0.75 (Fig. 3.7B). On the first and second day of the chill, mean predawn $F_v/F_m$ dropped about 50% to $0.38 \pm 0.03$ and $0.34 \pm 0.02$, respectively. Ten days later, $F_v/F_m$ in leaves that had experienced the chill had recovered to pre-chill levels ($0.69 \pm 0.06$) (Fig. 3.7B). A new cohort of 46 leaves selected along three transects had predawn $F_v/F_m$ levels similar to leaves that had expanded prior to the chill (Fig. 3.7B).

The quantum yield of photochemical energy dissipation ($\Phi_P$) was measured simultaneously with leaf gas exchange (Fig. 3.7C, circles). Prior to the chill, the pattern in $\Phi_P$ mirrored the pattern of $A_{net}$, declining from $0.12 \pm 0.007$ on May 5 (Jul = 125) to $0.04 \pm 0.003$ on the first day of the chill (Jul = 129) (Fig. 3.7C). After the chill, $\Phi_P$ recovered to levels about 40% greater than those measured prior to the chill.

To determine how the non-photochemical component of the absorbed light energy

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**Figure 3.7 (following page)** – Air temperature and fluorescence parameters measured during the 2010 chill event. (A) Mean (solid line), minimum and maximum (dashed lines) daily air temperature at Harrow meteorological station. (B) Mean ($\pm$ se) maximum efficiency of PSII measured before dawn (predawn $F_v/F_m$) on the same leaves followed in Fig. 3.3C–E (circles). Triangle is the mean $\pm$ se of predawn $F_v/F_m$ measured on 46 representative healthy leaves. (C) The quantum yield of photochemical energy dissipation ($\Phi_P$) on the same leaves followed in Fig. 3.3C–E (circles) and six unmarked representative leaves (triangles). (D) The mean ($\pm$ se) quantum yield of non-photochemical energy dissipation ($1-\Phi_P$) of the same six unmarked leaves measured in (C) partitioned into regulated dark-reversible dissipation ($\Phi_{Reg}$), combined constitutive and fluorescence dissipation ($\Phi_{I,CON}$) and dissipation related to the inactivation of PSII ($\Phi_{NF}$) such that $\Phi_P + \Phi_{Reg} + \Phi_{I,CON} + \Phi_{NF} = 1$. (E) The mean ($\pm$ se) ratio of $\Phi_{Reg}$ to $\Phi_{NF}$ (crosses).
was partitioned, a separate set of leaves was measured on May 6, 9, 10, and 30 in 2010 (Jul = 126, 129, 130, 150). $\Phi_P$ measured on these days was similar to $\Phi_P$ in the gas exchange leaves (Fig. 3.7 C, triangles). The total non-photochemical component of energy dissipation ($\Phi_{NP} = 1 - \Phi_P$) was stable at 0.92–0.95 prior to and during the two-day chill, but dropped to 0.77 ± 0.02 twenty days later when $A_{net}$ was high (not shown). The regulatory component of non-photochemical energy dissipation ($\Phi_{Reg}$) accounted for the bulk of $\Phi_{NP}$, remaining between 0.6 and 0.7 on all measurement days (Fig. 3.7 D). The combined fluorescence and constitutive components ($\Phi_{f,CON}$) were also stable at 6 to 9% of the total energy (Fig. 3.7 D). The component of $\Phi_{NP}$ that accounts for the light-induced deactivation of PSII ($\Phi_{NF}$) declined from 0.20 on the second day of the chill to 0.03 twenty days later (Fig. 3.7 D). The decline in $\Phi_{NF}$ relative to $\Phi_{Reg}$ accounts for the increase in energy available for photochemistry at the end of May (Fig. 3.7 C, E).

### 3.4.6 Climate analysis

To evaluate whether kudzu would be successful in other parts of Ontario, the probabilities of temperature events in Guelph, Barrie, and North Bay were estimated and compared to results from Leamington. The mean calculated date of budbreak for Leamington is May 14 (Jul = 134), 10–14 days earlier than that calculated for Guelph (May 26 (Jul = 146)), Barrie (May 28 (Jul = 148), or North Bay (May 25 (Jul = 145)) (Table 3.3). In Leamington, kudzu could emerge any time in May, but predicted earliest emergence in the other three locations was in mid-May. In Leamington, Guelph, and Barrie, kudzu should emerge by the first week in June, but in North Bay, budbreak could occur as late as Jun 14 (Table 3.3).

The frequency of years with a severe chill similar to that observed in Leamington in May 2010 ($T_{min} = 0–2^\circ C$, Table 3.1 A) was calculated for the 30 day period after the mean and earliest dates of budbreak (Table 3.4). Temperatures did not drop below 2°C after the latest date of emergence at any station. Kudzu emerging on the mean budbreak date
Table 3.3 – Estimated mean date (and Julian Day) of kudzu emergence over the 10 year period 2002–2011. In each year, the date was estimated as the first day after May 1 when the 10 day rolling mean temperature exceeded 13°C. Climate data provided by Environment Canada (www.climate.weatheroffice.gc.ca). The stations were Harrow CDA (∼ 20 km W of Leamington, 42.03°N, 82.90°W, elevation = 191 m.a.s.l., Climate ID = 6133362), Fergus MOE (∼ 20 km NW of Guelph, 43.70°N, 80.38°W, 396 m.a.s.l., ClimateID = 6142402), Barrie-Oro (for 2004–2011 only, 44.48°N, 79.55°W, 289 m.a.s.l., ClimateID = 6117700), Barrie WPCC (for 2002–2003 only, 44.38°N, 79.69°W, 221 m.a.s.l., ClimateID = 6110557), and North Bay A (46.36°N, 79.42°W, 370 m.a.s.l., ClimateID = 6085700).

<table>
<thead>
<tr>
<th>Location</th>
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</thead>
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<td>May 15 (135)</td>
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<td>May 17 (137)</td>
<td>Jun 6 (157)</td>
</tr>
<tr>
<td>North Bay</td>
<td>May 25 (145)</td>
<td>May 13 (133)</td>
<td>Jun 14 (165)</td>
</tr>
</tbody>
</table>
would have experienced three or five years (in the 10 year period 2002–2011) with severe chills in Barrie or North Bay, compared to two in Guelph and Leamington (Table 3.4 A). Chilling events lasted up to four days in Barrie compared to two days in Leamington and North Bay (Table 3.4 B). Separate sequential chilling events (Table 3.4 C) are unlikely after the mean budbreak date. Subzero temperatures (freezing events where $T_{\text{min}} = -2\text{–}0 ^\circ\text{C}$) occurred in one of the last ten years in Leamington and in Barrie (but not in Guelph), and in three of the last ten years in North Bay (Table 3.4 A). No station recorded temperatures below $-2 ^\circ\text{C}$ after the mean budbreak date (Table 3.4 A).

Chilling temperatures are much more common if kudzu emerges early, as it did in 2010 in Leamington (Table 3.1). $T_{\text{min}}$ was between 0–2 $^\circ\text{C}$ in six to eight of the last ten years at all four stations, but the chilling events lasted longer in Barrie (six days) than in Leamington (four days) or at the other two stations (three days, Table 3.4 B). Chilling events in Barrie and North Bay are more likely than those in Guelph or Leamington to last more than one day (Table 3.4 A, B). Separate sequential chilling events were more than twice as likely in North Bay (occurring in five of the ten years) than at the other three stations (Table 3.4 C). The number of years with freezing events did not differ substantially among the four stations (Table 3.4 A), but multi-day freezing events were more likely in North Bay (occurring in three years out of ten) than in the other three locations (Table 3.4 B). Leamington has had no multi-day freezing events in the last ten years. Barrie is the only station that recorded a multi-day freezing event below $-2 ^\circ\text{C}$ (Table 3.4 B). In North Bay, two of the last ten years had separate sequential freezing events within the 30 days following the estimated date of early budbreak; the other stations had none.

The probability of temperatures falling above or below a physiological threshold on a given day was estimated to assess how kudzu might perform in the first 30 days after budbreak. The potential for maximum growth rates did not differ by more than 10% (3 days) in any of the four locations. For shoots emerging near the mean budbreak date,
Table 3.4 – Number of years in the 10 year period 2002–2011 where damaging temperatures occurred (A) on at least one day, (B) on > 1 consecutive day, or (C) as separate events.

Values for each station are calculated for the 30 day period after the mean and earliest dates of budbreak (Table 3.3). Minimum temperatures ($T_{\text{min}} < 2^\circ$C) did not occur after the latest estimated date of budbreak at any station. Station details and data sources are given in Table 3.3. An event where $T_{\text{min}} = 0–2^\circ$C would have effects on kudzu life history similar to those caused by the 2010 chill reported here. The effects of colder temperatures are presumably more severe. Past frequencies are interpreted as future probabilities in the text.

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<th>Barr</th>
<th>NBay</th>
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Table 3.4 – Number of years in the 10 year period 2002–2011 where damaging temperatures occurred (A) on at least one day, (B) on > 1 consecutive day, or (C) as separate events. Values for each station are calculated for the 30 day period after the mean and earliest dates of budbreak (Table 3.3). Minimum temperatures ($T_{\text{min}} < 2^\circ$C) did not occur after the latest estimated date of budbreak at any station. Station details and data sources are given in Table 3.3. An event where $T_{\text{min}} = 0–2^\circ$C would have effects on kudzu life history similar to those caused by the 2010 chill reported here. The effects of colder temperatures are presumably more severe. Past frequencies are interpreted as future probabilities in the text.
growth rates will be suppressed to ~25% of its maximum potential (< 5 cm d\(^{-1}\)) about 5–10% of the time at all stations. High growth rates (> 10 cm d\(^{-1}\)) would be possible on about one-third (26–34%) of the days following mean budbreak. Early emerging shoots will spend one or more days growing slowly in Leamington (21%) and North Bay (26%), than in Guelph (17%) or Barrie (14%) (Table 3.5). The probability of high growth rates in early emerging plants is lowest in Leamington (14%) and between 19–22% in the other three locations (Table 3.5), but this difference only amounts to about 2 days. Late emerging kudzu plants will grow at least 10 cm d\(^{-1}\) about 40% of the time in all four locations. Thus, kudzu would be expected to grow between 5–10 cm d\(^{-1}\) roughly 60% the time, independent of budbreak date, at all four stations.

The potential for photosynthesis in shoots emerging near the mean budbreak date is marginally higher in Leamington than it is in Barrie (Table 3.5). Leaves would experience temperatures conducive to high \(A_{\text{net}}\) \((T_{\text{min}} \geq 12.5^\circ\mathrm{C}, \text{Fig. 3.6})\) about 10% more often in Leamington than in Barrie; differences are smaller for the other two stations. Differences in the probability that \(T_{\text{min}} < 10^\circ\mathrm{C}\), which reduces \(A_{\text{net}}\) to ~25% of its maximum capacity (Fig. 3.6), are similarly small among stations, ranging from 35% in Leamington to 42% in Barrie. Any differences among stations disappear if kudzu emerges early. \(A_{\text{net}}\) will be depressed about 60% of the time, and high rates will be possible 18–24% of the time in all locations. If kudzu emerges late, however, leaves will achieve high \(A_{\text{net}}\) more frequently in Leamington than in the other three locations. High \(A_{\text{net}}\) will be possible 72% of the time in Leamington, compared to 60% in North Bay, 46% in Guelph and 40% in Barrie. The probability of low \(A_{\text{net}}\) is similar in Leamington (10%) and North Bay (15%), but its substantially higher in Guelph (28%) and Barrie (36%).
Table 3.5 – Probability (%) of temperatures occurring on any given day in the 30 day period after the estimated mean date of budbreak, and after the earliest and latest dates (Table 3.3). Values are calculated using data from the 10 year period 2002–2011 for each station. Station details and data sources are given in Table 3.3. The significance to life history is inferred from Figs. 3.5 and 3.6. Probabilities highlighted in bold are at least 10% different (amounting to at least a 3 day difference in the 30 day period) from probabilities in Leamington.

<table>
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<td>max growth &gt; 10 cm d$^{-1}$</td>
<td>27</td>
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<td>$T_{\text{min}} &lt; 10 ^\circ C$</td>
<td>$A_{\text{net}} &lt; 5 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$</td>
<td>35</td>
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<tr>
<td>$T_{\text{min}} \geq 12.5 ^\circ C$</td>
<td>$A_{\text{net}} &gt; 10 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$</td>
<td>43</td>
</tr>
</tbody>
</table>
3.5 Discussion

This study evaluated whether chilling temperatures limit kudzu’s current northern range limit. Kudzu performance was assessed in two contrasting years. In 2010, kudzu emerged early (May 4) and experienced a severe chill \( T_{\text{min}} = 0.8^\circ\text{C} \); in 2011, kudzu emerged near the mean budbreak date (May 14) and experienced a mild \( T_{\text{min}} = 5.6^\circ\text{C} \) chill. The effects of the 2010 chill were more severe than that in 2011, but neither event had detectable lasting effects. By early June in both years, the canopy had closed and effects of the chill were no longer apparent (Fig. 3.1). Growth and photosynthesis rates were similar to summer levels (Chapter 4). The 2010 chill killed an estimated 50% of exposed leaves but only about 15% of stems (Fig. 3.3). Mortality was negligible after the 2011 chill. The 2010 chill impaired development of shoots and leaves that were immature during the chill, but these effects did not prevent canopy closure by early June. That year, the population went on to produce viable seed and survive the following winter, showing that any setbacks from the severe chill were not sufficient to substantially interrupt kudzu life history. Based on qualitative observation that co-occurring species had similar visual symptoms of chill damage (in 2010, not shown) and delays in phenology (in 2011, Fig. 3.1), chilling temperatures also did not appear to have impaired kudzu’s competitive ability relative to other species. In both years, kudzu was able to form a dense canopy that suppressed competing vegetation, despite chilling conditions.

Recovery was probably facilitated by the ability of kudzu growth and photosynthesis to quickly respond to moderations in temperature. The fastest growing stems increase their growth rate by 5 cm d\(^{-1}\) with every 10°C increase in maximum temperature (up to 35°C) (Fig. 3.5). There is also no detectable lag effect induced by exposure to chilling temperatures (Table 3.2). Growth rates 10 or 11 days following the chill were identical to what would be expected if there were no lag effect of the chill (Table 3.2). Kudzu shoots therefore seem to retain the potential to exploit warm days following exposure to chilling temperatures down to 1°C, provided the stem tips are not killed.
Photosynthesis is also sensitive to temperature. $A_{\text{net}}$ appears to be inhibited by morning temperature 12.5°C or below on the day of measurements (Fig. 3.6). Below a morning minimum temperature of 10°C, $A_{\text{net}}$ does not exceed 5 $\mu$mol m$^{-2}$ s$^{-1}$, even when measured at warm temperatures. Low $A_{\text{net}}$ during the severe chill in 2010 was associated with a 50% reduction in $\Phi_P$, which is a measure of the amount of light energy used by photochemistry, and in $F_v/F_m$, which reflects the proportion of functional PSII (Hendrickson et al., 2003; Allen and Ort, 2001; Long et al., 1994). Reductions in both of these parameters indicate that kudzu was photoinhibited by the combined effects of chilling temperatures and high irradiance (Allen and Ort, 2001). Leaves that experienced the severe 2010 chill had recovered both $F_v/F_m$ and $\Phi_P$ 10 days later (Fig. 3.7), so leaves were able to repair or replace damaged photosystems despite temperatures that were still in the chilling range (i.e., $T_{\text{min}} < 10$°C, Table 3.1) (Powles, 1984). Photoinhibition analysis shows that, prior to the chill, leaves had a greater proportion of deactivated PSII than leaves that were measured 20 days later (as evidenced by $\Phi_{\text{Reg}}/\Phi_{\text{NF}}$) (Fig. 3.7). This could be due to protective deactivation of PSII, or to the failure to repair damaged PSII (Öquist and Huner, 2003; Grennan and Ort, 2007). If caused by the former, the first kudzu leaves may be able to protect the photosynthetic apparatus from chilling-induced photoinhibition. Taken together, the evidence indicates that kudzu experiences some mortality, but no immediate delays in growth or photosynthesis, due to springtime chilling at its current range limit.

### 3.5.1 Implications for kudzu’s northern range limit

Kudzu is clearly able to tolerate chilling events typical of Leamington, but springs should be colder in more northern areas, where kudzu is not present. To evaluate whether spring conditions north of Leamington could prevent kudzu’s establishment, the past 10 years (2002–2011) of the climate record was analyzed for Leamington, Guelph, Barrie and North Bay, spanning 650 km of southern and central Ontario. Early spring temperatures
at the three northern locations stay cool longer than in Leamington, leading to a 1–2 week delay in the estimated date of budbreak (Table 3.3). The budbreak estimates reported here rely on the assumption that kudzu budbreak is associated with a thermal threshold; this assumption is well-founded (Schwartz et al., 2006). The global trend towards earlier budbreak is widely cited as evidence that global warming is causing ecological changes (Parmesan and Yohe, 2003; Walther et al., 2002; Cleland et al., 2007). The substantial difference in emergence date between the two years of this study suggests that kudzu is also responding to thermal cues (Table 3.1). The identified temperature threshold is imprecise, however, because stems had already begun to emerge, and the 10 day window used for the rolling mean is a crude measure of warmth necessary for budbreak (Schwartz et al., 2006). Nevertheless, the relative differences between sites and years reported here should still hold.

The occurrence of at least one severe chilling event in the 30 days following mean budbreak \( T_{\text{min}} = 0–2^\circ \text{C} \) is more likely in Barrie and North Bay than in Guelph and Leamington. Whether this will pose a barrier to kudzu establishment depends on the cumulative effect of successive years with severe chills. The Leamington kudzu patch appeared to recover completely from the severe chill in 2010; if so, then it should not matter if such a chill happens every year. Chilling events are also longer in duration in Barrie (but not in North Bay). A longer chill might cause more damage, but new shoots emerge continuously over a 60 days period following budbreak (Tsugawa et al., 1987, 1993b), so if one cohort is killed, there is opportunity for a new flush. Also, a survey of shoot and bud densities conducted during the chill in 2010 revealed that 16% of all of the shoots were protected from extreme temperatures under the leaf litter (Chapter 1). If kudzu maintains this cohort of fresh protected buds during canopy development, then it should have some ability to regrow a canopy even if most of the existing shoots are damaged. Overwintering kudzu stems maintain dormant buds and a substantial ability to branch, so there is potential for recovery there too (Tsugawa et al., 1987, 1988, 1993b).
While kudzu can recover from a single severe chill, separate successive chilling events in the same year would pose a much greater challenge to kudzu success. This is because kudzu cannot completely regrow its canopy multiple times. Early growth of vines draws heavily on starch stores in stems and roots (Bailey, 1939); if they are not sufficiently large (because of an unproductive season the previous year, or a particularly long winter), they may not be able to support yet another flush. There is good evidence that the performance of kudzu in one season depends on carbon stores accumulated the previous year (Terrill et al., 2003). Kudzu plots in Alabama were harvested 0–3 times in a growing season and total dry matter was measured the following season. Plots that were harvested three times in one year had 1/3 the total dry matter of plots that weren’t harvested (Terrill et al., 2003). A single harvest reduced dry matter by 30%; two harvests halved it (Terrill et al., 2003). Instructions on kudzu cultivation also reflect the need to restrict the number of times the canopy regenerates. In the early twentieth century, southeastern U.S. farmers were instructed to harvest kudzu for hay no more than twice per season, and only after the canopy had fully established (Bailey, 1939). Kudzu forage crops can also be destroyed by overgrazing (Bailey, 1939). This evidence indicates that there may be a carbon cost of regrowing a canopy following a single chill, but the cost grows as damage increases and, in particular, as stores are depleted by successive grow-outs. Successive chills are likely only if kudzu emerges early, and then only in North Bay is the probability higher than in Leamington (Table 3.4 C). So while successive chills would compromise kudzu’s ability to establish, they do not seem to be associated with kudzu’s current range limit.

Subzero temperatures may pose more of a barrier to kudzu establishment. In Leamington, there is a 10% probability of at least one day of $T_{\text{min}} = -2–0^\circ\text{C}$ after mean bud-break, and 40% if kudzu emerges early, so kudzu can probably tolerate the occasional mild freeze. The timing of the freeze will matter, however. This is because above-ground overwintering stems, which are responsible for the bulk of canopy development (Tsugawa
et al., 1987), lose their freezing tolerance at the start of the growing season. About one month after budbreak in 2010, stems collected in Leamington were killed by a 4 hour exposure to temperatures close to zero (Chapter 2, Fig. B.2). Such an event would be catastrophic; kudzu would only be able to regenerate from buds protected belowground or under leaf litter. During the chill in 2010, there were plenty of buds on stems protected by leaf litter and soil (ca. 60 m⁻², data not shown), but some of these might be endangered by a long freeze, which occurs in 10–30% of the years elsewhere in Ontario (Table 3.4). Sustained subzero temperatures could cool soils to a point that endangers the survival of belowground tissues; deacclimated belowground stems and roots also die at subzero temperatures above about −3 °C (Chapter 2, Figs. 2.5, B.3). It is unclear when deacclimation occurs, but the onset of rapid growth is usually associated with deacclimation (Kalberer et al., 2006), so it might happen within a few days before budbreak. The probability of a single day of subzero temperatures after the mean budbreak date is 30% in North Bay and 10% in Leamington and Barrie (Guelph had none) (Table 3.4). Multi-day events, and successive events in a single year, are likely only in North Bay after the mean budbreak date. Thus, late spring freezes probably exclude kudzu, but they do not explain kudzu’s current northern range limit.

In contrast to chilling and freezing events, the probability of temperatures favorable for growth are equally likely in all four locations following then mean budbreak date (Table 3.5). Kudzu emerging near the mean budbreak date will grow faster than 10 cm d⁻¹ 26–34% of the time, and less than 5 cm d⁻¹ 5–11% of the time, in all four locations. Temperatures too cool for growth will not prevent kudzu from establishing up to North Bay. There are, however, fewer days in Barrie and Guelph, were \( A_{\text{net}} \) is above 10 \( \mu \text{mol} \text{ m}^{-2} \text{ s}^{-1} \), and there are more days where \( A_{\text{net}} \) is below 5 \( \mu \text{mol} \text{ m}^{-2} \text{ s}^{-1} \); in North Bay, mornings are only marginally cooler than in Leamington (Table 3.5). In Barrie in particular, the cumulative effects of lower photosynthesis rates over a shorter
growing season would result in smaller carbon stores, which would reduce kudzu’s ability to recover from spring chills or freezes the following year. Cumulative chills may also reduce kudzu’s ability to compete with other species, or to resist attacks by pathogens or herbivores.

The results of this study show that spring chilling events do not unequivocally explain kudzu’s current range limit. Kudzu could certainly establish in other parts of southern Ontario with climates similar to Leamington. This includes prime agricultural and viticultural lands on Lake Erie’s north shore, and in the Niagara Peninsula. Kudzu could also establish in other parts of southern Ontario, including Guelph, another agricultural seat. Establishment in North Bay and Barrie is less likely because the greater probability of more than one chilling or freezing event per year should have a cumulative effect on growth in subsequent years. Barring extreme low temperature events, however, kudzu should be able to achieve the same high growth rates observed in Leamington at other locations in Ontario. This is largely due to its apparently high thermal threshold for budbreak, which increases the probability of favourable temperatures once it starts growing. A similar strategy allows the boreal C₄ grass *Muhlenbergia glomerata* to persist north of 60 °C (Kubien and Sage, 2003). Farmers also employ methods to delay budbreak in crops like grapevine for this reason (Poling, 2008). The success of this strategy for kudzu and other species depends on the ability to trade off the benefits of avoiding damaging temperatures, and the carbon cost of a shorter growing season.

That cost, however, is rapidly becoming smaller. Global warming advanced the date associated with initial understory growth ("first leaf") by up to 3 days per decade between 1970 and 2000 in southern and central Ontario, compared to a northern hemisphere mean of 1.2 days per decade (Schwartz et al., 2006). In the northeastern U.S., this indicator should continue to advance by 2.1 days per decade if emissions are not reduced (Hayhoe et al., 2006). That said, the number of days between "first leaf" and the last spring freeze (a "damage" index), is also changing; both are shifting earlier, but at different
rates, so the potential for damage could stay the same, or worsen (Schwartz et al., 2006). Nevertheless, a longer growing season, coupled with an expected shift towards higher maximum temperatures (Meehl et al., 2000; Hayhoe et al., 2006), should increase kudzu’s ability to recover from damaging events in the spring.

The hypothesis that spring chilling restricts kudzu expansion is supported, but not at its current range limit. There is still at least a ca. 300 km difference between where kudzu is currently, and where the results of this study show that spring chilling (or freezing) might be limiting. This supports the alternative hypothesis that kudzu is limited by dispersal rather than climate (Chapter 2). If so, Canada’s southern agricultural belt is already vulnerable to kudzu invasion. Within a few decades, that region of vulnerability will also include central Ontario as the climate is moderated. If kudzu is dispersal limited, Ontario will need to prevent its spread through education and monitoring programs in the most vulnerable areas. This study lends further support to the notion that an understanding of the relationship between physiology and life history can facilitate regulatory decisions and help minimize the costs of managing species invasions.
Chapter 4

The temperature response of growth and photosynthesis in kudzu (Pueraria montana var. lobata) and its significance for future range expansion

This chapter is the product of collaborations with Rowan F. Sage, Kate Henbest and Ferit Kocacinar. Rowan Sage contributed ideas and helped with the writing. Kate Henbest and Ferit Kocacinar contributed data (noted in the figure captions).

4.1 Abstract

Kudzu (Pueraria montana var. lobata) in an Asiatic vine that is invasive in North America. Kudzu has migrated northward as winters have warmed, suggesting that low temperatures are a barrier to kudzu range expansion. Winter freezing mortality and devel-
opmental delays due to late spring chills do not explain this biogeographic pattern. Here, the impact of cool summer temperatures on growth and photosynthesis is evaluated. The temperature response of kudzu growth and photosynthesis was measured in plants growing in cool and warm conditions in growth cabinets and at a field site at the northern range limit. There was no evidence of a decline in growth or photosynthesis at the range limit. Growth rates were fast, responding within hours to changes in temperature, and peaking at 22 cm d\(^{-1}\). Most growth occurs during the day when temperatures are warm, so cool nights have little impact on growth rates. Leaves grown at 17–22\(^\circ\)C have 20–25\% lower net CO\(_2\) assimilation rates when measured at the temperature optimum compared to leaves grown at 25–31\(^\circ\)C. There was also no apparent shift in the thermal optimum, indicating that leaves cannot acclimate photosynthesis to cool conditions. Temperatures cool enough to impair photosynthesis do not occur often at the current range limit, and leaves tend to be 2–5\(^\circ\)C warmer than air temperature on cool days, mitigating potential impacts of cool air temperatures. A comparison of summer conditions in Leamington to three other locations in Ontario shows that summer temperatures are somewhat less favourable for maximum growth and photosynthesis north of its current range limit, potentially reducing kudzu’s viability. Nevertheless, kudzu has the potential to do well in other parts of southern Ontario, making regulation of kudzu a priority in these areas.

4.2 Introduction

Kudzu (\textit{Pueraria montana} var. \textit{lobata} (Willd.) Ohwi) is an Asiatic vine that is invasive in North America. Kudzu was promoted throughout the southeastern U.S. for its ability to prevent soil erosion, but that practice ceased when it was recognized as an invasive weed (Bailey, 1939; Forseth and Innis, 2004). One consequence of kudzu infestations is the emission of air pollutants; kudzu leaves emit isoprene and soils under kudzu stands emit nitrous oxides (Hickman et al., 2010; Sharkey and Loreto, 1993). The most deleterious
effect of kudzu infestations, however, is reduced biodiversity (Forseth and Innis, 2004). Trees and other plants starve when layers of kudzu leaves block sunlight necessary for photosynthesis (Shurtleff and Aoyagi, 1985; Forseth and Innis, 2004). A rapid growth rate (up to 30 cm d$^{-1}$) and climbing growth habit help kudzu cover telephone poles, buildings, and vehicles with blankets of vines, creating a public nuisance (Blaustein, 2001; Shurtleff and Aoyagi, 1985).

Kudzu is no longer confined to the southeastern U.S. states. Since 1970, kudzu has tracked the $-20 ^\circ$C minimum winter temperature isocline northward to invade New York City, two New England states, all but one of the Great Lakes states, and southern Ontario (Chapter 2). The correlation between minimum winter temperature and kudzu’s northern limit have inspired predictions that kudzu will continue to move north with climate change (Chapter 2, Sasek and Strain, 1990; Follak, 2011; Ziska et al., 2011; Wolfe et al., 2008; Jarnevich and Stohlgren, 2009). The strength of these projections depends on whether climate is limiting kudzu’s distribution in some way. Previous work has ruled out both cold winters and spring chills as obvious determinants of its current distribution limit (Chapters 2, 3). Kudzu is often described as a warm-climate species that requires long hot summers, but its distribution in North America and in Asia extends into the cool temperate zone, where summers can be short and cool (Miller and Edwards, 1983; Sasek and Strain, 1990; Shurtleff and Aoyagi, 1985; van der Maesen, 2002; Martyn, 1992). If cool summers are limiting kudzu’s distribution, it should not be able to achieve high growth or photosynthesis rates at its northern range limit.

Kudzu is known for its fast growth rate, which is a key feature of its invasiveness (McClain et al., 2002). Kudzu performs poorly in the shade, so it must grow fast enough to capture available sunlight before other plants can (Tsugawa et al., 1985). It is unclear whether kudzu stems can maintain high growth rates at the lower temperatures that occur at its northern limit. Growth rates in all plants are influenced by temperature (Lambers et al., 1998), but the relationship between growth and temperature is not always
clear. This is in part because the underlying metabolic components of growth respond to temperature in different ways (Loveys et al., 2002; Criddle et al., 1997). For example, fast-growing species have lower respiration rates at low measurement temperatures (relative to high temperatures) than slow-growing species (Loveys et al., 2002). One consequence of this phenomenon may be that fast or slow growth rates are more predictive of the temperature response of growth than is the species’ thermal origin (Loveys et al., 2002; Tjoelker et al., 1999). A first step, then, in assessing whether cool summer temperatures define kudzu’s northern range limit, is to determine the relationship between temperature and growth rate. If most of stem elongation happens in the afternoon when temperatures are highest (Berman and Dejong, 1997; Cremer, 1976), early morning lows would have little impact on overall growth rates even if growth is depressed at cool temperatures.

Another way low summer temperatures could define kudzu’s northern range limit is by depressing its photosynthesis rate. To be competitive in a cool temperate climate, kudzu must be able to assimilate enough carbon to maintain high growth rates during the growing season, which depends on achieving a high net CO$_2$ assimilation rate ($A_{net}$) (Larcher, 2003; Chapin et al., 1990). $A_{net}$ is commonly depressed by short-term exposure to cool temperatures (Sage and Kubien, 2007). However, some plants have the ability to acclimate (i.e., biochemically adjust $A_{net}$) when exposed to longer periods of cold (Atkin and Tjoelker, 2003; Sage and Kubien, 2007; Berry and Bjorkman, 1980). This is true of many species including *Populus balsamifera* (Silim et al., 2010), lowland (but not alpine) *Plantago* sp. (Atkin and Tjoelker, 2003), and several evergreen shrubs (Berry and Bjorkman, 1980). If the net photosynthesis rate of kudzu acclimates to cool temperatures, then cool northern summers are less likely to be a limiting factor at its northern distribution.

There is some evidence that kudzu photosynthesis and respiration can acclimate to low temperatures. Photosynthetic acclimation to low temperature is usually accompanied by a shift in the temperature optimum towards the cooler growth temperature (Berry and Bjorkman, 1980; Sage and Kubien, 2007). Plants from Maryland grown in
cool conditions (either in a greenhouse at 19°C or outside in Madison, WI, where the mean June–August temperature was 20.7°C, had a slightly lower thermal optimum for photosynthesis (25°C) than plants grown in the field in Maryland or in Georgia (mean June–August temperature: 24.7°C and 25.7°C, respectively), where the thermal optimum was 25–30°C (www.ncdc.noaa.gov, Sharkey and Loreto, 1993; Wechsler, 1977; Forseth and Teramura, 1987). If kudzu can acclimate photosynthesis to cool growth temperatures, then it may be better adapted to succeed beyond its current range limit than previously thought.

In this study, I test the hypothesis that cool temperatures restrict kudzu growth and photosynthesis. I do this by characterizing the temperature response of growth and photosynthesis in kudzu plants growing at cool and warm temperatures in the field and in the lab. I then evaluate whether cool temperature restrictions might determine its northern distribution limit.

### 4.3 Methods and Materials

#### 4.3.1 Field study

To determine the temperature response of growth and photosynthesis in the field, measurements of leaf gas exchange and stem elongation rates were conducted under cool summer (June 2011) and warm summer (July 2011) conditions in Leamington, ON (42.02°N, 82.74°W). The site covers 120 × 50 m of a steep south-facing embankment on the north shore of Lake Erie. The site is bordered by a cultivated field, the lakeshore, and forest (Fig. 1.2). Environmental conditions were logged at the Environment Canada weather station in Harrow, ON, Canada, about 20 km west of the site (42.03°N, 82.9°W, elevation = 191 m.a.s.l., Climate ID = 6133362, WMO ID = 71298, TC ID = XHA) (www.climate.weatheroffice.gc.ca).
4.3.2 Controlled acclimation experiment

To evaluate the temperature response of growth and photosynthesis in controlled conditions, measurements of leaf gas exchange and stem elongation were conducted on potted kudzu plants growing in growth cabinets. Plants were third-generation clones of plants collected in 2007 from naturally-growing populations in Montrose, New York (41.25°N, 73.94°W) and Vicksburg, Mississippi (32.09°N, 90.93°W). Plants were started by rooting nodes of vines attached to older plants in 20 L pots filled with soil (20% Triple-mix, 50% Pro-mix, 10% perlite, 20% sand). Plants were grown either outside in a roof-top garden at the Earth Sciences Centre at the University of Toronto, or in a glasshouse (16/8 h, 26/18°C day/night, ≥ 1000 µmol−1 m−2 s−1 light) prior to transfer to a growth cabinet. Plants were kept trimmed, and were fertilized twice weekly with a 50/50 mix of 24–8–16 Miracle-Gro (Scotts Canada Ltd, Mississauga, Ontario) and 30–10–10 evergreen fertilizer (Plant Prod, Brantford, Ontario), supplemented with full-strength Hoagland’s. Two plants per population per treatment were grown in each of two identical growth cabinets (Conviron PGR15, Winnipeg) set to 26°/22°C, 14/10 h day/night, and a photon flux density (PFD) of 500–700 µmol−1 m−2 s−1. After at least two weeks of acclimation, temperatures in the cabinets were changed so that leaf temperatures were 31°/22°C (warm) and 22°/14°C (cool). These treatments are similar to the conditions during the June (cool) and July (warm) field study in Leamington (Table 4.1). Plants were rotated daily throughout the experiment and gas exchange measurements were performed on leaves that developed at the growth temperature.

4.3.3 Controlled stem elongation experiment

To determine the response of growth to temperature under controlled conditions, eight young kudzu plants were grown from small crowns collected in Leamington, ON in May 2011. Plants were grown in a roof-top garden at the Earth Sciences Centre at the Uni-
versity of Toronto in 20 L pots filled with soil (20% Triple-mix, 50% Pro-mix, 10% perlite, 20% sand), watered twice daily and fertilized twice weekly with 24–8–16 Miracle-Gro (Scotts Canada Ltd, Mississauga, Ontario) and 30–10–10 evergreen fertilizer (Plant Prod, Brantford, Ontario) supplemented with 1 mM MgSO$_4$ and 5 mM CaNO$_3$. For each temperature treatment, two plants were covered with clear plastic bags and transferred to a room where the first seven nodes of five stems were threaded through a small port in the side of a temperature-controlled chamber (Model S–16–8200, Thermotron Industries, Holland, Michigan) set to 25°C. Plants outside the chamber were at room temperature and dimly illuminated with cool-beam flood lamps (< 200 µmol m$^{-2}$ s$^{-1}$); stems inside the chamber were not illuminated. After the growth rate had stabilized (ca. 4 hours), the chamber temperature was changed at 2.5°C h$^{-1}$ to one of eight target temperatures (5–40°C) for 15 hours. After this, the chamber was returned to 25°C over 30 min, and the plants were returned to the roof-top garden where they recovered for at least two days. Stem length was measured every two hours up to the beginning of the 15 hour target period, at the end of the target period, and then periodically after the treatment to ensure growth rates returned to normal. This procedure was replicated twice in August 2011.

4.3.4 Growth measurements

To measure stem elongation rates in all experiments, stems were marked with a loose cable tie below the sixth or seventh node. Shoot length was measured from the marked node to the shoot tip with a flexible seamstress tape. Growth rate for each shoot was calculated as described previously (Chapter 3).

At the field site in Leamington, the elongation rate of 20 stems was measured every 1–2 days during cool summer (early- to mid-June) and warm summer conditions (July). Stems were located along a single transect that ran east–west through the middle of the patch. Because preliminary observations show that large stems grow faster than small
stems (Fig. C.3), stems with a range of diameters (measured with digital calipers (±0.01 mm, VWR, Mississauga, Ontario) below the sixth node) were selected.

To determine the diurnal pattern of growth, a second set of stems along the same transect was marked and measured at 2 h intervals. For this set, ten large (diameter > 4 mm) stems were chosen because they should be the fastest growing and most temperature responsive (Figs. 3.5, C.3). For all field measurements, the same two sets of stems were followed during both study periods in June. New sets of stems were selected for the July study period because the June set were overgrown and could not be found.

In the controlled acclimation experiment, the growth rate of 3–6 stems per plant were monitored over the duration of the experiment. Stems that broke or exceeded 1 m in length were replaced with younger stems.

Table 4.1 – Summary of air temperatures prior to, and during, two bouts of measurements in June, and one in July. Daily air temperature measurements were logged at Harrow CDA meteorological station in Harrow, Ontario (www.climate.weatheroffice.gc.ca). Mean temperature during each 4–10 day time period is shown with mean minimum and mean maximum temperatures in parentheses. Absolute minimum and maximum temperatures are shown in square brackets.

<table>
<thead>
<tr>
<th>Month in 2011</th>
<th>10 d period prior to measurements, °C</th>
<th>4–7 d period during measurements, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early June (May 30–Jun 2)</td>
<td>17.3 (12.3, 22.2)</td>
<td>22.1 (17.2, 27.0)</td>
</tr>
<tr>
<td>Mid-June (Jun 12–18)</td>
<td>21.3 (15.7, 26.8)</td>
<td>18.5 (13.2, 23.6)</td>
</tr>
<tr>
<td>Mid-July (Jul 19–22)</td>
<td>24.6 (19.8, 29.3)</td>
<td>28.2 (22.4, 33.8)</td>
</tr>
</tbody>
</table>
4.3.5 Field gas exchange measurements

In 2011, the temperature response of net CO$_2$ assimilation rate ($A_{\text{net}}$) of leaves acclimated to cool summer conditions in early- and mid-June was compared to leaves acclimated to hot summer conditions of mid-July (Table 4.1). All measurements were conducted on the third fully expanded leaf using an open-type portable gas exchange system (LI–6400, LI–COR, Lincoln, NE, USA). Conditions in the gas exchange cuvette were saturating light (800–1400 $\mu$mol$^{-1}$ m$^{-2}$ s$^{-1}$) and an ambient CO$_2$ concentration of 390 $\mu$mol$^{-1}$ mol. The leaf–to–air vapor pressure deficit (VPD) was 1–1.5 kPa below 35 $^\circ$C. Above 35 $^\circ$C, VPD was 1.5–4 kPa. Temperature control in the cuvette was facilitated using a water jacket (LI–6400–88, LI–COR, Lincoln, NE, USA) with the Peltier block fans disabled. The water jacket was connected to a gravity-fed water line connected to two 80 L coolers. Low leaf temperatures were achieved by filling the bath with ice water and by surrounding the cuvette with bags of ice water and a styrofoam box. High leaf temperatures were achieved by filling the water bath with water heated with a propane camp stove and by sunshine on the cuvette. Air was humidified at high temperature by adding 10 ml water to the soda lime and by bubbling incoming air through a carbon filter partially immersed in warm water (LI–COR Biosciences, Inc., 2004). Leaves were light-adapted at 20–22 $^\circ$C and 1400 $\mu$mol$^{-1}$ m$^{-2}$ s$^{-1}$ for at least 30 min before leaf temperature was reduced to the lowest measurement temperature. Leaves were equilibrated to each measurement temperature for at least 30 min before a measurement was taken. Measurements were conducted in 5 $^\circ$C increments. If $A_{\text{net}}$ did not fully recover at 20–22 $^\circ$C following low measurement temperatures, a new leaf was chosen.

To observe diurnal patterns of $A_{\text{net}}$ \textit{in situ}, gas exchange was measured in three marked leaves on June 13–14 (6 leaves total). Measurements of instantaneous $A_{\text{net}}$ were taken every two hours with the LI-COR gas exchange system described above. Prior to each measurement, ambient photon flux density (PFD) was measured by holding a quantum sensor (LI–189, LI–COR, Lincoln, Nebraska) level at arm’s length. Air temperature was
measured with a 40-gauge Type T thermocouple suspended at 3 m height in a large radiation shield attached to a data logger ((VL-1700, Veriteq Instruments, Richmond, BC). At each time point, cuvette conditions were set to match the leaf environment as closely as possible. Cuvette light conditions were matched to incident PFD. Leaf temperature was measured by touching the shaded side of the leaf with a 40–gauge Type T thermocouple attached to an instant-read datalogger (LI–1000, LI–COR, Lincoln, NE). Block temperature of the LI-6400 was set to achieve that leaf temperature in the cuvette. The VPD inside the cuvette was the difference between the vapor pressure (VP) in the intercellular spaces and that of the ambient air. The VP of the air was measured in the sample cell of the open cuvette, while the VP in the leaf was read from a saturation vapor pressure table using measured leaf temperature and assuming 100% RH in the intercellular spaces (Sharkey, 1985). The VPD was always within 0.3 kPa of the target VPD. If leaf conditions changed, the process was restarted.

The response of \( A_{\text{net}} \) to changes in VPD was measured on June 18 and on July 19–20, 2011. The June measurements were taken with the LI–COR gas exchange system described above, while the July measurements were taken with a second portable gas exchange system (GFS–3000, Heinz Walz, Effeltrich, Germany). Leaf temperature in both systems was 30°C, with an ambient CO\(_2\) concentration of 390 \( \mu \text{mol}^{-1} \text{mol} \) and saturating light (1400 \( \mu \text{mol}^{-1} \text{m}^{-2} \text{s}^{-1} \)). Leaves were equilibrated to a VPD of 0.8–1.0 kPa before increasing the VPD stepwise to 4.0 kPa. At each target VPD, the leaf was permitted to acclimate for at least 10 min prior to measurement.

### 4.3.6 Lab gas exchange measurements

Leaf gas exchange of plants growing in controlled conditions was measured with the LI–6400 gas exchange system described above. Temperature response curves were measured under saturating light (800–1200 \( \mu \text{mol}^{-1} \text{m}^{-2} \text{s}^{-1} \)) and a CO\(_2\) concentration of 400 \( \mu \text{mol}^{-1} \text{mol} \). Leaf-to-air vapor pressure deficit was 1–2.2 kPa at 30°C and below. Above 30°C,
VPD was allowed to increase to 6 kPa at 40°C. Leaves were acclimated for at least 40 min at each temperature prior to measurements. Temperatures were increased in 4°C increments from 22° to 42°C, returned to 22°C and then decreased to 10°C. Temperature control was facilitated by placing the whole plant and the gas exchange system inside a growth cabinet and adjusting the cabinet temperature to within 5°C of the target leaf temperature.

4.3.7 Climate analysis

To evaluate if cool summer temperatures limit kudzu’s northward range expansion in Ontario, the climate in Leamington was compared to the climate in Guelph (277 km NE from Leamington), Barrie (410 km NE), and North Bay (659 km NE). Daily weather data was obtained from Environment Canada weather stations for the 9 year period 2002–2010 (www.climate.weatheroffice.gc.ca). The stations were Harrow CDA (∼20 km from Leamington, 42.03°N, 82.90°W, elevation = 191 m.a.s.l., Climate ID = 6133362), Fergus MOE (∼20 km east of Guelph and the closest station with data, 43.70°N, 80.38°W, elevation = 396 m.a.s.l., Climate ID = 6142402), Barrie-Oro (for 2004–2011 only, 44.48°N, 79.55°W, 289 m.a.s.l., Climate ID = 6117700), Barrie WPCC (for 2002–2003 only, 44.38°N, 79.69°W, 221 m.a.s.l., Climate ID = 6110557), and North Bay A (46.36°N, 79.42°W, 370 m.a.s.l., Climate ID = 6085700).

To estimate whether kudzu would be able to achieve high rates of photosynthesis and growth north of Leamington, the probability of temperatures falling above or below physiological temperature thresholds were calculated for June–August in the 10 year period 2002–2011. Temperature thresholds relevant to kudzu success were determined based on the temperature response of photosynthesis and growth. The thresholds were: $T_{\text{max}} < 15$°C, which slows growth to $< 5 \text{ cm d}^{-1}$; $T_{\text{max}} < 22$°C, which slows photosynthesis rates to $< 12 \mu\text{mol}^{-1} \text{ m}^{-2} \text{ s}^{-1}$; and $T_{\text{max}} = 25–35$, which is the temperature range for optimal growth and photosynthesis (Figs. 4.1, 4.5).
The probability of a damaging chill event in a given year was estimated by counting the number of years (out of ten) where $T_{\text{min}}$ met the following criteria on at least one day: $T_{\text{min}} = 0\text{–}2^\circ\text{C}$, which may cause partial mortality of aboveground stems (Fig. B.2 D) and require substantial canopy regrowth; $T_{\text{min}} = 2\text{–}5^\circ\text{C}$, which may be damaging if fast summer growth rates come at the expense of reduce cold acclimation (Chapter 3; Kalberer et al., 2006). The intensity of summer cold events was estimated by counting the maximum number of days per year that met the criteria. R Project software v. 2.12.1 was used for all analyses (R Development Core Team, 2011).

4.4 Results

4.4.1 Environmental conditions

Leaves measured in early or mid-June developed at a mean temperature of 17.3$^\circ\text{C}$ and 22.1$^\circ\text{C}$, respectively (Table 4.1). These temperatures are below kudzu’s photosynthetic optimum of 25–30$^\circ\text{C}$. Conditions during the June measurement periods were similar (Table 4.1) so they were pooled to represent cool-season growth. Leaves measured in mid-July developed at 24.6$^\circ\text{C}$, with mean minimum and maximum temperatures of 19.8$^\circ\text{C}$ and 29.3$^\circ\text{C}$, respectively (Table 4.1). The measurement period in July coincided with a heat wave in southern Ontario, with temperatures peaking at 37.8$^\circ\text{C}$. In all cases, measurements took place no more than three days following rain (not shown) to minimize the effects of water stress.

4.4.2 Response of growth to temperature in the field

The elongation rate of 20 stems was measured daily in the field in Leamington, Ontario. Mean stem elongation rates ranged from $3.9\pm0.4$ to $8.6\pm0.6$ cm d$^{-1}$ in June and $11.5\pm0.7$ to $12.8\pm0.9$ cm d$^{-1}$ in July (Fig. D.1). The temperature variable that best described stem elongation rates was the mean maximum temperature since the previous measurement
Figure 4.1 – Stem elongation rate as a function of mean maximum temperature over the 1–2 day period pertaining to the measurement. Open symbols are the overall means, solid symbols are means of the fastest quintile (20%) and crossed symbols are the means of the slowest quintile. Circles represent growth rates in Leamington, Ontario (Fig. D.1); squares represent growth rates from the controlled acclimation experiment (Fig. D.2). Regression lines are fit to the field data only. Regression lines are extrapolated to the $x$-axis to estimate the temperature where growth is zero. The regression for the upper and lower quintiles are $y = 0.79x - 7.52$ (adj. $R^2 = 0.77$) and $y = 0.30x - 4.37$ (adj. $R^2 = 0.57$). The regression fit to the overall mean was $y = 0.59x - 7.3$, adj. $R^2 = 0.40$ ($F_{1,208} = 142.5$, $P(< |F|) \leq 0.001$). The $x$-intercepts of the extrapolated lines predict zero growth will occur between 9.5°C and 14.5°C. Kate Henbest collected these data under the supervision of H. Coiner.
Figure 4.2 – Mean elongation rate of stems measured in the field at Leamington, Ontario as a function of stem diameter. Points are the growth rate of each measured stem, averaged over all of the days that it was observed in June (open symbols, $N = 5$ days) and July (closed symbols, $N = 2$ days). Data from both months are fit simultaneously with the linear model: 

$$\text{Mean rate} = \beta_0 + \beta_0 \text{Diameter} + \beta_0 \text{Month},$$

where $\beta_0 = -3.0$, $\beta_1 = 2.7$, and $\beta_2 = 4.7$, adj. $R^2 = 0.73$. ANOVA results are shown in Table D.2. Kate Henbest collected these data under the supervision of H. Coiner.
(Table D.1, Fig. 4.1). The maximum growth rate measured was 15.6 cm d\(^{-1}\) in June and 22.5 cm d\(^{-1}\) in July (Fig. D.1). At 19°C, the lowest maximum temperature that occurred in the field (not shown), the upper 20% of stems grew 7.5 cm d\(^{-1}\) (Fig. 4.1). The \(x\)-intercepts of the extrapolated models predict zero growth will occur between 9.5 and 14.5°C (Fig. 4.1). The fastest-growing stems were also the thickest stems measured (Fig. 4.2). Stems of all sizes grew 4.7 cm d\(^{-1}\) faster in July than in June (\(F_{1,56} = 51.2\), \(P(< |F|) \leq 0.001\), Fig. 4.2, Table D.2).

To determine the diurnal pattern of growth, a second set of 10 large (> 4 mm in diameter) stems was measured every two hours at the field site in Leamington in June and July 2011 (Fig. 4.3). Air temperatures were 10–27°C in June, and 20–38°C in July (Fig. 4.3A, C; Table 4.1). On days that followed a day of measurements (June 13, 14, 18, and July 21), the first point represents the average growth rate during the previous night. On the other days (June 17 and July 22), the first point represents growth during the previous two hours (Fig. 4.3B, D). Growth is generally low in the night, even when nights are warmer, peaking when temperatures peak in the afternoon. Maximum diurnal growth rates were similar in both months, reaching 1.9 cm h\(^{-1}\) in June and 1.7 cm h\(^{-1}\) in July (equivalent to 46 and 41 cm d\(^{-1}\), respectively). Daily mean growth rates were 0.4–0.6 cm h\(^{-1}\) (9–15 cm d\(^{-1}\)) in June and 0.3–0.5 cm h\(^{-1}\) (7–12 cm d\(^{-1}\)) in July. On June 17, and July 20, a midday depression in growth rates was observed, which was followed by a surge in mid- to late-afternoon.

4.4.3 Response of growth to controlled temperature treatments

To determine the response of growth to temperature under controlled conditions, elongating stems were exposed to a randomized series of temperatures in a temperature-controlled chamber (Model S–16–8200, Thermotron Industries, Holland MI, USA). Plants were at room temperature. Growth slowed from an average of 1.7 mm h\(^{-1}\) (equivalent to 4.0 cm d\(^{-1}\)) at 20°C to 0.4 mm h\(^{-1}\) at 15°C and 0.1 mm h\(^{-1}\) at 10°C (equivalent
Figure 4.3 – Hourly air temperature at Harrow meteorological station (42.03°N, 82.9°W, www.climate.weatheroffice.gc.ca) in (A) June and (C) July 2011 and corresponding diurnal measurements of stem elongation rate in (B) June and (D) July. Mean ± se (open circles) and maximum (closed diamonds) growth rates are shown for 12 marked stems in Leamington, Ontario. Kate Henbest collected these data under the supervision of H. Coiner.
Figure 4.4 – The temperature response of stems exposed to treatments in a controlled temperature chamber (Model S–16–8200, Thermotron Industries, Holland, Michigan). Plants were at room temperature. Open circles are the mean ± se of two trials, (N = 10–20 stems on 4 plants). Solid diamonds are the maximum measured rate at each temperature. Regression lines are the upper quintile (dashed) and mean (solid) rates of field data shown in Fig. 4.1. *Data collected by K. Henbest under the supervision of H. Comer.*
to 1.0 and 0.2 cm d$^{-1}$, respectively). This is consistent with the zero-growth thresholds of 9.5–14.5$^\circ$C predicted by field measurements (Fig. 4.1). At 30$^\circ$C, mean elongation rates were 8.4 cm d$^{-1}$. At temperatures below 30$^\circ$C, the results of this controlled study are consistent with the temperature response of growth observed in the field (Fig. 4.1). Above 30$^\circ$C, however, elongation rates began to decline in the potted plants, while they continued to increase (up to 35$^\circ$C) in the field-grown plants. In the controlled acclimation experiment, there were no differences between plants from Mississippi and New York (not shown), so results were pooled. Growth rates of plants grown in the warm treatment (31$^\circ$/22$^\circ$C) were also lower than field values, while plants grown in a cool treatment (22$^\circ$/14$^\circ$C) grew at a similar rate to field plants (Fig. 4.1).

### 4.4.4 Response of photosynthesis to temperature

To evaluate the acclimation potential of photosynthesis in kudzu, the response of $A_{\text{net}}$ to temperature was measured in the field and in the lab under cool and warm conditions (Fig. 4.5). In the controlled acclimation experiment, there were no significant differences between plants from Mississippi and New York (not shown), so the results were pooled.

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**Figure 4.5 (following page)** – The temperature response of photosynthesis in field-grown (A–D) and potted (E–H) plants. Shown are $A_{\text{net}}$ (A, E) along with stomatal conductance (B, F), the ratio of intercellular to atmospheric CO$_2$ ($C_i/C_a$) (C, G), and the leaf–to–air vapor pressure difference (VPD) (D, H). Open circles are measurements taken on plants growing in cool field conditions in June 2011 (A–D) or at 22/14$^\circ$C day/night in growth cabinets (E–H). Solid circles are measurements taken on plants growing in warm field conditions in July 2011 (A–D) or at 31/22$^\circ$C day/night in growth cabinets (E–H). The temperature response curves contain points from 4 leaves. They also include single points taken from 3–4 additional leaves used for light and CO$_2$ response curves (not shown), and VPD curves (Fig. 4.7).
Chapter 4. Summer growth and photosynthesis

Net CO₂ assimilation rate, µmol CO₂ m⁻² s⁻¹

Stomatal conductance, mol H₂O m⁻² s⁻¹

Air-leaf vapor pressure difference, kPa

Measurement temperature, °C
Temperature response curves from the field and lab were similar. The thermal optimum for warm-grown plants ranged from 22–27 °C to 35–37 °C (Fig. 4.5A, E). In cool-grown plants the temperature response curve was flatter. Optimum temperatures started at cooler temperatures of 18–25 °C, but the upper limit of the range was similar to that of warm-grown plants (Fig. 4.5A, E). Maximum $A_{\text{net}}$ was similar between the field and the lab: 21.9 $\mu$mol m$^{-2}$ s$^{-1}$ in July (Fig. 4.5A), and 22.7 $\mu$mol m$^{-2}$ s$^{-1}$ in the warm growth cabinet treatment (Fig. 4.5E). In the cool treatments, maximum $A_{\text{net}}$ was 18.2 in June (Fig. 4.5A), and 16.8 $\mu$mol m$^{-2}$ s$^{-1}$ in the cool growth cabinet treatment (Fig. 4.5E). Thus, maximum net CO$_2$ assimilation rates are about 20–25% lower at the temperature optimum in cool vs. warm-grown leaves (Fig. 4.5A, E). The evidence for a shift in thermal optimum is equivocal. In the field-grown plants, there is a suggestion of a 2–3 °C shift in the thermal optimum when plants are grown in cool vs. warm conditions, but this shift is absent in the lab results (Fig. 4.5A–E). There is no evidence that cool-grown leaves have higher $A_{\text{net}}$ at low measurement temperatures. The difference between $A_{\text{net}}$ measured in the warm and cool treatments disappears at 22 °C and below in the field results and at 18 °C and below in the lab results (Fig. 4.5A, E). In contrast, at measurement temperatures > 35 °C, net CO$_2$ assimilation rates in cool-grown plants in the field is about 40% that in warm-grown plants (Fig. 4.5A, E). Differences are smaller in the plants grown in a controlled environment, with cool-grown plants experiencing about a 20% reduction in $A_{\text{net}}$ relative to warm-grown plants. Stomatal conductances were similar in all treatments, averaging 0.17–0.19 mol H$_2$O m$^{-2}$ s$^{-1}$ except for warm-grown plants in the field, which had variable stomatal conductance (Fig. 4.5B, F). The ratio of intercellular to ambient CO$_2$ ($C_i/C_a$) was similar among all treatments (Fig. 4.5C, G).

### 4.4.5 Diurnal response of photosynthesis

The diurnal response of leaf photosynthesis in situ was measured on two similar days in June in Leamington (one representative leaf from each day is shown in Figs. D.3, D.4).
Figure 4.6 – Difference between leaf temperature and air temperature as a function of the difference between incident (PFD$_i$) and ambient (PFD$_a$) photon flux density. PFD$_a$ was measured by holding a quantum sensor (LI–189, LI–COR, Lincoln, Nebraska) level at arm’s length. PFD$_i$ was measured by holding a quantum sensor parallel to the leaf surface. Leaf temperature was measured by touching the shaded side of the leaf with a 40–gauge Type T thermocouple attached to an instant-read data logger (LI–1000, LI–COR, Lincoln, NE). Air temperature was measured with a 40–gauge Type T thermocouple suspended at 3 m height in a large radiation shield attached to a data logger (VL–1700, Veriteq Instruments, Richmond, BC). Points are individual measurements, taken at two-hour intervals, of 3 leaves on each of 2 days in June 2011 in Leamington, Ontario. Negative values of PFD$_a$–PFD$_i$ occur when leaves turn towards the sun, positive values occur when leaves turn away from the sun. The solid line is a linear regression that shows no relationship between leaf temperature and leaf angle. The dashed line is where leaf and air temperature are equivalent. The full diurnal responses of two of the six leaves are shown in Figs. D.3 and D.4.
Figure 4.7 – The response of (A) net CO$_2$ assimilation rate, (B) stomatal conductance, and (C) $C_i/C_a$ to changes in leaf–to–air vapor pressure difference (VPD), measured at a constant leaf temperature of 30°C in Leamington, Ontario in June (open symbols, N=1) and in July (closed symbols, N=3) 2011. Lines are linear (A) or loess (local) (B, C) regression fits to all of the data. The regression line is (A) is $y = 26.5 - 4.7$, adj. $R^2 = 0.81$.

*Ferit Kocacinar contributed the July data to this figure.*
Three of the leaves maintained $C_i/C_a$ at around 0.6 throughout the day (e.g., Fig. D.3), indicating that the stomates were open; the other three closed their stomates midday (e.g., Fig. D.4). All leaves were consistently 2–5°C warmer than air temperature, even when oriented away from the sun (Fig. 4.6). The decoupling of leaf temperature from incident radiation suggests that latent heat exchange was offsetting warming effects of increased solar radiation.

### 4.4.6 Response of photosynthesis to VPD

To test whether small increases in leaf–to–air vapor pressure difference (VPD) cause reductions in $A_{\text{net}}$, the response of photosynthesis to VPD at 30°C was measured in Leamington on June 18 and on July 19–20, 2011 (Fig. 4.7). $A_{\text{net}}$ responds immediately to increases in VPD, dropping by 4.7 µmol$^{-1}$ m$^{-2}$ s$^{-1}$ for every 1.0 kPa increase in VPD (Fig. 4.7A). Maximum $A_{\text{net}}$ was achieved at 0.88 kPa. $A_{\text{net}}$ was halved at 3.29 kPa (Fig. 4.7A). Stomatal conductance declines to a similar degree as $A_{\text{net}}$ at higher VPD, causing calculated $C_i/C_a$ to change little with increasing VPD (Fig. 4.7).

### 4.4.7 Climate analysis

To evaluate whether kudzu would be successful in more northern parts of Ontario where it is not currently present, the probability of summer (July–August) temperature events in Guelph, Barrie, and North Bay were estimated and compared to probabilities in Leamington. In Leamington, $T_{\text{max}}$ was less than 15°C just 4% of the time, so kudzu has a 96% probability of growing more than 5 cm d$^{-1}$ on average (Fig. 4.1). In Guelph and Barrie, there are about 6–8 more days (7–9% of 90 d) where growth would be slow in summer. In North Bay, $T_{\text{max}}$ is less than 15°C nearly 1/5 of the time (Table 4.2).

The three northern locations had more days with with air temperatures that were less favourable for photosynthesis than Leamington. $T_{\text{max}}$ falls below 22°C 20–25% of the time in Guelph and Barrie, compared to 10% of the time in Leamington; North
Table 4.2 – Probability (%) of temperatures occurring on any given day in June–August. Climate data were provided by Environment Canada (www.climate.weatheroffice.gc.ca) in the 10 year period 2002–2011. The stations were Harrow CDA (≈ 20 km from Leamington, 42.03°N, 82.90°W, elevation = 191 m.a.s.l., Climate ID = 6133362), Fergus MOE (≈ 20 km from Guelph, 43.70°N, 80.38°W, 396 m.a.s.l., ClimateID = 6142402), Barrie-Oro (for 2004–2011 only, 44.48°N, 79.55°W, 289 m.a.s.l., ClimateID = 6117700), Barrie WPCC (for 2002–2003 only, 44.38°N, 79.69°W, 221 m.a.s.l., ClimateID = 6110557), and North Bay A (46.36°N, 79.42°W, 370 m.a.s.l., ClimateID = 6085700). The significance to life history is inferred from Figs. 4.1 and 4.5.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Significance to life history</th>
<th>Leam</th>
<th>Guel</th>
<th>Barr</th>
<th>NBay</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{\text{max}} &lt; 15^\circ C$</td>
<td>max growth &lt; 5 cm d$^{-1}$</td>
<td>4</td>
<td>11</td>
<td>13</td>
<td>18</td>
</tr>
<tr>
<td>$T_{\text{max}} &lt; 22^\circ C$</td>
<td>$A_{\text{net}} &lt; 12 , \mu\text{mol}^{-1} , \text{m}^{-2} , \text{s}^{-1}$</td>
<td>10</td>
<td>21</td>
<td>24</td>
<td>34</td>
</tr>
<tr>
<td>$T_{\text{max}} = 25$–$35^\circ C$</td>
<td>optimum $A_{\text{net}}$ and growth</td>
<td>67</td>
<td>51</td>
<td>48</td>
<td>38</td>
</tr>
</tbody>
</table>

Table 4.3 – Number of years in the 10 year period 2002–2011 where potentially damaging temperatures occurred on at least one day in June–August. The maximum number of days per year (d y$^{-1}$) is given in parentheses. Minimum temperatures ($T_{\text{min}}$) below 0°C did not occur at any station. Station details and data sources are given in Table 4.2. The significance to life history is inferred from Fig. B.2 and Chapter 3.

<table>
<thead>
<tr>
<th>Variable $T_{\text{min}}$</th>
<th>Significance to life history</th>
<th>Leam</th>
<th>Guel</th>
<th>Barr</th>
<th>NBay</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–2°C</td>
<td>Partial mortality</td>
<td>0</td>
<td>0</td>
<td>1 (2)</td>
<td>3 (1)</td>
</tr>
<tr>
<td>2–5°C</td>
<td>Canopy damage</td>
<td>2 (2)</td>
<td>7 (3)</td>
<td>5 (10)</td>
<td>7 (4)</td>
</tr>
</tbody>
</table>
Bay is colder still (Table 4.2). In Leamington, temperatures are in the optimum range ($T_{\text{max}} = 25\text{–}35$) for both growth and photosynthesis 67% of the time, compared to about 50% of the time in Guelph or Barrie (Table 4.2). North Bay has optimum temperatures just 38% of the time (Table 4.2).

There is a higher likelihood of potentially damaging cold events in Barrie and North Bay than in Leamington or Guelph. $T_{\text{min}}$ does not drop close to zero ($0\text{–}2\,^o\text{C}$) in the two southern locations, but it did in one year out of the last ten in Barrie, and in three of the last ten years in North Bay (Table 4.3). Milder chill events that could damage the canopy ($T_{\text{min}} = 2\text{–}5\,^o\text{C}$) occurred in two of the last ten years in Leamington. These events are also more likely in Guelph, Barrie, and North Bay, occurring in 5–7 of the last 10 years (Table 4.3). The intensity of these events is substantially greater in Barrie, where chilling temperatures occurred for as many as 10 days in a single year (9% of the time, Table 4.3).

4.5 Discussion

The goal of this study was to evaluate whether cool summer temperatures could determine kudzu’s northern range limit. If summer temperatures limit kudzu expansion, then there could be evidence that growth and photosynthesis rates decline in northern populations (Sexton et al., 2009). No such declines are observed in Leamington, Ontario population, located at kudzu’s northern range limit. Kudzu growth rates responded strongly and rapidly (within 1–2 hours) to changes in temperature (Fig. 4.3). The fastest 20% of stems increased their growth rates by nearly 8 cm d$^{-1}$ with every 10$^o$C increase in maximum temperature up to 35$^o$C (Fig. 4.1). Maximum growth rates in Leamington (up to 20 cm d$^{-1}$) were similar to growth rates reported in southern populations (e.g., 19 cm d$^{-1}$ in Georgia), where kudzu is a problem (Forseth and Innis, 2004; Wechsler, 1977). They were slower than the maximum reported growth rates of 30 cm d$^{-1}$ (Forseth
and Innis, 2004; Shurtleff and Aoyagi, 1985), but still fast enough to overtop surrounding vegetation, bring down a tree, and require active management in the neighbouring field (H.A. Coiner, personal observation).

In June, growth rates accelerate in the morning, peak in the afternoon, and are slow at night (Fig. 4.3). Similar diurnal elongation patterns are seen in peach (Berman and Dejong, 1997), and *Eucalyptus regnans* (Cremer, 1976). These patterns are less clear in July. Stem elongation rates slow to ≤ 1 cm d\(^{-1}\) at 15°C and below, but these temperatures occur mainly at night, when kudzu does not grow (Figs. 4.1, 4.3, 4.4). The diurnal patterns are not due to circadian rhythm, because fast growth rates were achieved at night in the controlled growth experiment (Fig. 4.4). Moreover, the fastest measured growth rate in the diurnal dataset occurred after a nighttime low of 12°C, (13 Jun, Fig. 4.3B), so typical night-time lows are unlikely to suppress overall growth rates. Atypical lows are also unlikely to dramatically restrict growth; stems acclimated to 28/20°C day/night temperatures recovered from a 2 day exposure to 10/5°C within 3 days (Appendix A). Thus, seasonally cool temperatures in summer at Leamington do not prevent kudzu from exploiting daytime highs.

In contrast, photosynthesis rates at measurement temperatures greater than 20°C are impaired by development at cool growth temperatures (17–22°C) relative to leaves that developed at warm growth temperatures (25–31°C) (Fig. 4.5). Near the temperature optimum, \(A_{\text{net}}\) was reduced by 20–25% in cool- vs. warm-grown leaves (Fig. 4.5). These reductions at moderate to high measurement temperatures were not compensated for by increased performance at low measurement temperatures as \(A_{\text{net}}\) was identical in cool- and warm-grown plants below 20–22°C (Fig. 4.5). The range of temperatures where \(A_{\text{net}}\) is close to optimum values was broad in both warm- and cool-grown plants, but there was also no clear shift in the temperature optimum (Fig. 4.5). These patterns could result from a quantitative (i.e., uniform) increase in overall leaf protein in warm- vs. cool-grown leaves (Way and Sage, 2008b). There is, however, no evidence that cool-
grown leaves make qualitative changes (i.e., proportional shifts in allocation patterns) to enhance photosynthesis rates at cool measurement temperatures, since this would likely have shifted the thermal optimum (Fig. 4.5; Way and Sage, 2008b).

An inability to qualitatively acclimate photosynthesis should nevertheless have little impact on kudzu performance at its northern range limit because daytime leaf temperatures are not often in the limiting range. During most (90%) of the summer in Leamington, $T_{\text{max}}$ is greater than 22°C, which means that leaves are likely developing and operating within the range of optimum temperatures (Table 4.2, Fig. 4.5). When cool days do occur, leaves can be 2–5°C warmer than air temperature, so $A_{\text{net}}$ may still be close to the optimum (Fig. 4.6). Leaf temperature was not measured in July in Leamington, Ontario, but in July in Maryland, kudzu leaves can be as much as 4°C cooler than air temperature because of steep leaf angles and transpirational cooling, thereby avoiding high temperature depressions of $A_{\text{net}}$ (Forseth and Teramura, 1986, 1987). The ability to decouple leaf temperature from air temperature can allow a species to extend its distribution beyond what might otherwise be expected. For example, a C₄ grass, which requires leaf temperatures upwards of 25°C to compete with C₃ vegetation, has extended its range into the boreal zone (48°N) by maintaining leaf temperatures warmer than air temperatures (Kubien and Sage, 2003). Kudzu’s ability to decouple leaf temperature from air temperature in both hot and cool climates, together with a broad thermal optimum range of photosynthesis, may therefore help explain why kudzu can maintain broad native and naturalized distributions despite limited acclimation potential.

Maximum photosynthesis rates ($A_{\text{net}}$) were at least 14 $\mu$mol m$^{-2}$ s$^{-1}$, even on mornings where the minimum temperature was as low as 12°C (not shown); the maximum rate measured was 22.5 $\mu$mol m$^{-2}$ s$^{-1}$ (Fig. 4.5). These rates are within the range of $A_{\text{net}}$ measured in more southern locations (11–27 $\mu$mol m$^{-2}$ s$^{-1}$, Figs. 4.5) (Wechsler, 1977; Sharkey and Loreto, 1993; Forseth and Teramura, 1987; Carter and Teramura, 1988a). Kudzu’s peak photosynthesis rates are similar to soybean (22 $\mu$mol m$^{-2}$ s$^{-1}$).
and tomato (25 $\mu$mol$^{-1}$ m$^{-2}$ s$^{-1}$), but slow compared to other fast-growing C$_3$ species, such as wheat (40 $\mu$mol$^{-1}$ m$^{-2}$ s$^{-1}$), sunflower (33–35 $\mu$mol$^{-1}$ m$^{-2}$ s$^{-1}$), and the weed *Chenopodium album* (45 $\mu$mol$^{-1}$ m$^{-2}$ s$^{-1}$) (Sage et al., 1987; Makino et al., 1988; Paul et al., 1992; Bunce, 2000). Kudzu may be able to compensate for its moderate $A_{\text{net}}$ by permitting light to penetrate to lower leaf layers with steep leaf angles. Light interception would be reduced in upper canopy leaves, but $A_{\text{net}}$ saturates at about 50% sunlight, so upper canopy leaves would still maintain high rates of photosynthesis, while increasing $A_{\text{net}}$ in lower canopy leaves (Fig. 4.6; Forseth and Teramura, 1986, 1987). Leaf expansion rates are correlated with stem elongation rates (Fig. D.5), so new leaves should be produced. Enhanced canopy-level carbon gain depends on the ability of kudzu to maintain a thick canopy; the leaf area index of kudzu is typically 3–4 m$^2$ m$^{-2}$ or greater (Tsugawa et al., 1993b; Wechsler, 1977). In Leamington, Ontario, kudzu’s rapid growth rates, high leaf area index, and its ability to maintain moderate values of $A_{\text{net}}$ at a range of temperatures should ensure that kudzu maintains high canopy-level rates of carbon gain. Thus, low temperature inhibition of photosynthesis is also not likely to be restricting kudzu success at its northern range limit.

### 4.5.1 Implications for kudzu’s northern range limit

There is evidence that the quality of the growing season declines to the north of Leamington. It is unlikely that kudzu would do well as far north as North Bay because temperatures are suboptimal for both growth and photosynthesis 62% of the time, compared to 33% of the time in Leamington (Table 4.2). This would impair kudzu’s ability to recover from the more severe chill events that also occur there (Table 4.3). Summer chilling events are also more frequent in Guelph and Barrie, but these events alone are unlikely to pose a substantial barrier to kudzu establishment. Kudzu has thrived in Leamington, where chills have occurred in two of the last ten years (Table 4.3). A combination of factors may, however, reduce kudzu’s performance in Guelph and Barrie. At
both stations, there are fewer days with temperatures in the optimum range for growth and photosynthesis, more days where growth will be slowed, and more days where photosynthesis may be suboptimal (Table 4.2). While the differences are small, if they occur in early summer, they may have a disproportionately large effect on kudzu’s success, because both daylength and solar insolation peak in June (Natural Resources Canada, 1984). A late budbreak in Guelph or Barrie, or a June chill followed by suboptimal temperatures, could prevent kudzu taking advantage of June’s long sunny days (Chapter 3). This could have a large impact on kudzu’s ability to do well in these areas.

### 4.5.2 Implications for species distribution modeling

The evidence that growth or photosynthesis do not decline at the range limit adds to the growing body of literature showing that declines in growth and photosynthesis are not often associated with range limits (Sexton et al., 2009; Schenk, 1996). Growth declines do not tend to determine range limits in trees (Schenk, 1996), or in *Lactuca serriola*, a weedy annual (Carter and Prince, 1985; Prince and Carter, 1985). *Mimulus* plants sampled from central and peripheral populations also do not differ in growth and photosynthesis rates (Angert, 2006). One possible explanation for this is that the physiological effects of suboptimal temperatures are more subtle than other types of low temperature events, such as freezing, or severe chills, which can have catastrophic consequences (Levitt, 1980; Sakai and Larcher, 1987). Rather than a threshold effect on survival, one might expect suboptimal temperatures to result in a slight decline in performance that may be difficult to detect in a short-term experiment (Sexton et al., 2009). Long-term transplant experiments may do a better job of detecting the smaller effects of suboptimal temperature on population viability.

The results of this study show that there is no strong evidence that cooler summer temperatures limit kudzu’s current range limit. There is, however, some evidence that summer conditions may become limiting within 200 km of kudzu’s current range limit,
especially when the effects of late spring chill or early fall frost are taken into account (Chapters 3, 5). Kudzu should nevertheless be able to succeed in other parts of southern Ontario with a climate similar to Leamington, such as the entire north shore of Lake Erie, and the Niagara Peninsula. These regions account for much of Ontario’s agricultural lands, and its lucrative wine industry, so regulations should be put into place to protect these lands from kudzu invasion. Kudzu may not be able to do well in the agricultural region around Guelph in the current climate, but this will change rapidly. Mean summer temperatures in the northeastern U.S. have already warmed 0.12°C per decade since 1970, accelerating from an average warming of 0.7°C between 1900–1999 (Hayhoe et al., 2006). Summer temperatures are expected to continue to warm 3°C by 2064 unless emissions are reduced (Hayhoe et al., 2006). In southern and central Ontario, most of the warming has been driven by increases in summer minimum temperatures, which have warmed 1–3°C since 1950 (Bonsal et al., 2001). There has been no consistent trend in maximum summer temperatures, so the greatest benefit to kudzu in the future may be a lower frequency of potentially damaging events (Table 4.3), which should remove barriers posed by extreme low temperature events in central Ontario. In the meantime, invasive species managers in southern Ontario have an opportunity to implement early detection and management procedures before kudzu becomes a problem.
Chapter 5

Autumn frost tolerance of kudzu (Pueraria montana var. lobata) and its significance for future range expansion

This chapter is the product of a collaboration with Rowan F. Sage, who contributed ideas and helped with the writing.

5.1 Abstract

Kudzu is an invasive Asiatic vine that is expanding its naturalized range northward in North America. Kudzu’s northward migration is correlated with minimum winter temperature, but an unambiguous physiological mechanism linking low temperatures and kudzu success has not been identified. Kudzu is believed to be sensitive to fall frost; if so, such events could restrict kudzu invasion in northern areas by shortening the growing season and reducing carbon assimilation and storage necessary for winter survival.
Here, the impacts of fall frosts on leaf mortality, growth and photosynthesis were studied in kudzu plants growing in a rooftop garden in Toronto, Ontario. Growth rates decline irreversibly the first time $T_{\text{min}}$ drops below 8°C. Net CO$_2$ assimilation rates also decline when $T_{\text{min}}$ drops below 5°C, but some leaves maintain modest photosynthesis rates ($A_{\text{net}} = 2-5 \, \mu\text{mol}^{-1} \, \text{m}^{-2} \, \text{s}^{-1}$) until killed by frost. Leaves can survive mild frosts ($-2-0^\circ\text{C}$), but the canopy is killed when temperatures drop below $-2^\circ\text{C}$. The results show that growth rates are sensitive to fall chilling, but that leaves persist—and continue photosynthesis—well past the first mild frost. Since kudzu does not appear to regulate leaf senescence like most temperate deciduous species, it can direct photosynthate to storage and the acquisition of cold tolerance until leaves are killed by frost. A comparison of the climate record in Leamington, Ontario, where kudzu is present, to three other locations in Ontario where kudzu is not currently present, shows that there is no strong evidence that fall frost restricts expansion into these areas.

5.2 Introduction

Kudzu (Pueraria montana var. lobata (Willd.) Ohwi) is an invasive Asiatic vine that is expanding its naturalized range northward in North America (Chapter 2; Sasek and Strain, 1990; Ziska et al., 2011). Kudzu is known for its ability to cover vast hectares of land with fast-growing vines that reduce local biodiversity, remove land from production, and create a costly public nuisance (Forseth and Innis, 2004; McClain et al., 2002). Infestations also degrade air quality through emissions of isoprene and nitrous oxides, the two precursors for the formation of ozone, an air pollutant (Hickman et al., 2010; Sharkey and Loreto, 1993). Kudzu is often characterized as a cold-sensitive species that grows actively until leaves and aboveground stems are killed by the first fall frost (Sasek and Strain, 1990; Carter and Teramura, 1988a; Mitich, 2000; Sorrie and Perkins, 1988).
Still, kudzu is successful in northern U.S. states and southern Canada, so it may not be as frost sensitive as these anecdotes imply.

The idea that kudzu is cold sensitive, along with the observation that kudzu’s northward movements correspond to warming winters, has led to the hypothesis that cold temperatures restrict kudzu’s northward range movement (Chapter 2, Sasek and Strain, 1990; Forseth and Innis, 2004; McClain et al., 2002). The mechanistic basis for this hypothesis remains unclear. Winter cold, the most obvious putative limiting factor, is not the cause, because overwintering kudzu stems survive temperatures colder than those normally observed at the range limit (Chapter 2). Suboptimal summer temperatures also do not explain the correlation. Summer growth rates respond within hours to warming even when nights are cool (Chapter 4). Leaves that develop at low temperatures have low photosynthesis rates, leaves do not often experience these temperatures at the current range limit (Chapter 4). There are also no large differences between summer temperatures at kudzu’s northern range limit and areas further north (Chapter 4).

Another hypothesis that could explain the correlation between kudzu’s northern range limit and minimum winter temperature, is that growing seasons are too short in northern areas because of springtime chilling and early fall frost. Kudzu can recover from a single harsh spring chill, but successive chills or frosts in the spring could compromise kudzu’s canopy development and weaken the population (Chapter 3). Canopy regrowth is costly, reducing kudzu biomass in subsequent years (Terrill et al., 2003). If kudzu is also sensitive to fall frost, the amount of time available for replenishing carbon stores would be even shorter. Kudzu is thought to grow “actively” until killed by frost (Sasek and Strain, 1990). This could be maladaptive in temperate climates because cold hardening and the translocation of canopy resources to winter stores usually starts when demand by other carbon sinks (such as shoot elongation or flowering) drops off (Chapin et al., 1990; Lennartsson and Ogren, 2002; Wardlaw, 1990). In this study, I assess the impact of fall frost on leaf mortality, kudzu growth rates, and photosynthesis.
Table 5.1 – Kudzu populations and sample sizes for each study year. Leaf senescence was studied in Fall 2006. Fall measurements of growth and photosynthesis were taken in 2008 and in 2009 and summer growth measurements (controls) were taken in 2010. The number of plants per population that were used in each study is shown. Population differences were only assessed in 2008 and 2009. In 2006, an additional two plants from an unidentified population were also used (not shown).

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<td></td>
</tr>
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<td></td>
</tr>
<tr>
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<td>3</td>
</tr>
<tr>
<td>LI</td>
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<td>40.81°N</td>
<td>73.41°W</td>
<td>2</td>
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<td>4</td>
<td>3</td>
</tr>
<tr>
<td>PA</td>
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<td>3</td>
<td>4</td>
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</tr>
</tbody>
</table>
5.3 Methods and Materials

5.3.1 Plant material

All plants were grown from young kudzu crowns collected from 3–9 naturalized populations (Table 5.1). Study plants were at least second-generation clones of field-collected plants except for plants from Leamington, Ontario, Frankfort, Michigan, and Huntsville, Alabama, which were first generation clones. Plants were grown in 20 L pots filled with 20% Triple-mix, 50% Pro-mix, 10% perlite, 20% sand in a rooftop garden at the Earth Sciences Center at the University of Toronto. Plants were watered as needed and fertilized twice weekly with 24–8–16 Miracle-Gro (Scotts Canada Ltd, Mississauga, Ontario) and 30–10–10 evergreen fertilizer (Plant Prod, Brantford, Ontario) supplemented with full-strength Hoagland’s solution or 1 mM MgSO$_4$ and 5 mM CaNO$_3$. Vines were staked and trimmed as needed. All plants were moved into the rooftop garden in late May where they were maintained until the start of the study.

A naturalized kudzu population in Leamington, Ontario (42.02°N, 82.74°W) was visited in November 2009 and 2010. The site is between 10 and 40 years old and located on a steep south-facing embankment on the north shore of Lake Erie. The site is confined on all sides by the lake, a cultivated field, and forest (Fig. 1.2 B).

5.3.2 Environmental conditions

Air temperature was logged at 30 min intervals by a rooftop weather station installed adjacent to the garden at the University of Toronto. Leaf temperatures were measured with 36-gauge thermocouples (Type T, Omega, Laval, QC) connected to a datalogger (VL–1700, Veriteq Instruments, Richmond, BC). Thermocouples were attached to the underside of leaves with porous medical tape.
5.3.3 Stem elongation rate

Stem elongation rate was measured in autumn 2008 and 2009 and compared to growth rates measured in summer 2010. On the first day of each study, three shoots per plant were marked with a loose cable tie below the sixth node and followed throughout the study. Shoot length was measured from the marked node to the shoot tip with a seam-stress tape. Growth rate for each shoot was calculated as the difference in length between sequential measurements, divided by the difference in time, which was calculated in decimal days. In the fall, measurements continued until no living stem tips were found. If a stem died, a new stem was marked and measured to maintain constant number of stems. Plants were the unit of replication.

5.3.4 Gas exchange and chlorophyll fluorescence

Simultaneous leaf gas exchange and chlorophyll fluorescence measurements were determined on leaves in fall 2008 and 2009. Net CO₂ assimilation rates \( A_{\text{net}} \) were measured using an open-type portable gas exchange system with an integrated fluorescence head (LI–6400 and 6400–40, LI–COR, Lincoln, Nebraska, U.S.A.). Conditions in the cuvette were saturating light (1400 \( \mu \text{mol} \text{ m}^{-2} \text{s}^{-1} \)) and an ambient CO₂ concentration of 395 \( \mu \text{mol} \text{ mol}^{-1} \) mol in 2008 and 375 \( \mu \text{mol} \text{ mol}^{-1} \) mol in 2009. Leaf-air vapor pressure deficit (VPD) was maintained between 1–1.9 kPa in 2008 and 1–1.5 kPa in 2009. Leaf temperature outside the gas exchange cuvette was measured using a 36–gauge thermocouple (Type T, Omega, Laval, QC) attached to an instant-read datalogger (LI–1000, LI–COR, Lincoln, Nebraska, U.S.A.) and used to set leaf temperature inside the gas exchange cuvette. \( A_{\text{net}} \) was measured on clear days, starting at least one hour after leaves were fully illuminated. Predawn \( F_v/F_m \) was measured on 1–3 leaves per plant in 2008 and on 1 leaf per plant in 2009. Plants \( (N = 12) \) were the unit of replication.

In 2008, two separate gas exchange experiments were done. On Sept 25, Oct 6, 7, and
10 (Julian Days (Jul) = 269, 280, 281, and 284), a new upper canopy leaf was selected each day for studying the partitioning of absorbed light energy into photochemical and non-photochemical components. One young, fully-expanded, healthy-looking leaf per plant was selected the day before measurements. Immediately following simultaneous $A_{\text{net}}$ and fluorescence yield measurements, a 3.3 cm$^2$ leaf disc was sampled from the leaf used for gas exchange and placed in a foil-wrapped petri dish. After 30 min dark recovery, fluorescence yield was remeasured and fluorescence parameters were calculated according to Kornyeyev and Holaday (2008). These measurements were destructive, so once growth stopped, a second experiment was started. On October 12–13 (Jul = 283–284) two leaves per plant with similar $A_{\text{net}}$ values were selected and followed for the remainder of the study. In 2009, 1–2 similar leaves per plant were followed through the entire study.

5.3.5 Leaf mortality

In 2006, the impact of frost on leaf viability was studied. To assess membrane leakage due to chilling or freezing disruption, a 1 cm$^2$ leaf disc was harvested and incubated in 5 ml ddH$_2$O for 24 hours. The electrical conductivity of the solution was measured with a hand-held conductivity meter (Ultrameter 4P, Myron L Company, Carlsbad, CA, USA). The samples were then boiled, re-equilibrated, and the total conductivity was measured. The proportion of cells damaged by the freezing treatment is the ratio of treatment conductivity to total conductivity, or relative conductivity (RC) (Hallam and Tibbits, 1988).

A second 2.6 cm$^2$ leaf disk was harvested from the same leaf to assess relative water content. This is an indicator of leaf dessication, which may occur if e.g., hydraulic conductivity is disrupted by cavitation. Relative water content (RWC) was calculated as

$$\text{RWC} = \frac{FW - DW}{TW - DW}$$

where $FW =$ fresh weight, assessed immediately after harvest, $TW =$ turgid weight,
following a 24 hour equilibration in dH$_2$O, and $DW =$ dry weight, assessed after dehydration at 60 °C to constant weight.

**Table 5.2** – Summary of air temperatures during the three study years at the Earth Science Center at the University of Toronto. Shown are the dates and temperatures of the 10 days (d) prior to the start of the study, and during the study. Mean air temperature is shown with mean minimum and mean maximum temperatures in parentheses. Absolute minimum and maximum temperatures recorded during the period are in square brackets. The onset of cold days in each year is summarized as the first day when $T_{\text{min}}$ drops below 2 °C; the total number of cold days is shown in parentheses.

<table>
<thead>
<tr>
<th>Year</th>
<th>10 d prior to study, °C</th>
<th>During study, °C</th>
<th>First day $T_{\text{min}} &lt; 2$ °C</th>
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<tr>
<td></td>
<td>(Total No. of days)</td>
<td></td>
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</tr>
<tr>
<td>2006</td>
<td>Sep 19–Sep 29</td>
<td>Sep 30–Nov 21</td>
<td>Oct 13</td>
</tr>
<tr>
<td></td>
<td>14.9 (10.7, 19)</td>
<td>8.72 (5.76, 12.3)</td>
<td>(8 d)</td>
</tr>
<tr>
<td></td>
<td>[6.4, 25.5]</td>
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</tr>
<tr>
<td>2008</td>
<td>Sep 15–Sep 25</td>
<td>Sep 26–Oct 31</td>
<td>Oct 21</td>
</tr>
<tr>
<td></td>
<td>17.2 (12.9, 21.9)</td>
<td>12.1 (8.5, 16.1)</td>
<td>(4 d)</td>
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<tr>
<td></td>
<td>[9.3, 26.5]</td>
<td>[−0.3, 27]</td>
<td></td>
</tr>
<tr>
<td>2009</td>
<td>Sep 6–Sep 16</td>
<td>Sep 17–Nov 1</td>
<td>Oct 14</td>
</tr>
<tr>
<td></td>
<td>20.2 (16.2, 25.1)</td>
<td>12.2 (9.1, 15.9)</td>
<td>(2 d)</td>
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<td></td>
<td>[11.4, 27.2]</td>
<td>[1.5, 28.6]</td>
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**5.3.6 Climate analysis**

To evaluate if cool fall temperatures limit kudzu’s northward range expansion in Ontario, the climate in Leamington was compared to the climate in Guelph (277 km NE from Leamington), Barrie (410 km NE), and North Bay (659 km NE). Daily weather
Figure 5.1 – Mean (solid line), maximum, and minimum (dashed lines) air temperature during the three study years. Temperatures were logged every 30 min at a weather station located adjacent to the rooftop garden on the roof of the Earth Sciences Centre at the University of Toronto. Arrows indicate the beginning and end of measurements.
data was obtained from Environment Canada weather stations for the 9 year period 2002–2010 (www.climate.weatheroffice.gc.ca). Nine years was used because data for the current year (2011) was not yet available. The stations were Harrow CDA (Section 3.3.2), Fergus MOE (∼ 20 km east of Guelph and the closest station with data, 43.70°N, 80.38°W, elevation = 396 m.a.s.l., Climate ID = 6142402), Barrie-Oro (for 2004–2011 only, 44.48°N, 79.55°W, 289 m.a.s.l., Climate ID = 6117700), Barrie WPCC (for 2002–2003 only, 44.38°N, 79.69°W, 221 m.a.s.l., Climate ID = 6110557) and North Bay A (46.36°N, 79.42°W, 370 m.a.s.l., Climate ID = 6085700).

The timing of fall cold events was assessed by determining the first day after September 1 in each year where $T_{\text{min}}$ dropped below 8°C, 5°C, or −2°C. These thresholds correspond respectively to the irreversible decline in maximum growth rate, the irreversible decline in $A_{\text{net}}$ to < 5 μmol m$^{-2}$ s$^{-1}$, and leaf mortality (see Section 5.4). The dates were averaged to give a mean date for each station. R Project software v. 2.12.1 was used for all analyses (R Development Core Team, 2011).

5.4 Results

5.4.1 Environmental conditions

Measurements began on Sept 30, Sept 26, and Sept 17 (Jul = 273, 269, and 260) in 2006, 2008, and 2009, respectively. Air temperatures prior to the beginning of the study were warmer in the years with earlier start dates (Table 5.2, Fig. 5.1). In 2006, the study lasted 53 days, until the last leaves had abscised, while in 2008 and 2009, the study was stopped when photosynthesis was less than 5 μmol m$^{-2}$ s$^{-1}$. Autumn temperatures stayed warm one week longer in 2008 than in the other two years, as indicated by the first day minimum air temperatures ($T_{\text{min}}$) dropped below 2°C (Table 5.2). More days below 2°C occurred in 2008 and 2006 than in 2009, but the overall temperature in 2008 and
2009 was similar (Table 5.2). Temperatures in 2006 were about 4°C colder, on average, during the study (Table 5.2, Fig. 5.1).

### 5.4.2 Leaf mortality

In 2006, the impact of frost on leaf viability was assessed. In that year, leaf temperature ($T_{leaf}$) dropped below zero on Oct 13 (Jul = 286), Oct 26 (Jul = 299), Nov 2–4 (Jul = 306–308), and Nov 21 (Jul = 325). On these days, standing water in a bucket next to the plants formed a layer of ice, but air temperatures only dropped below 0°C on Nov 3 and Nov 21 (Fig. 5.2 B). On all other days when $T_{leaf}$ was subzero, $T_{min}$ was between 0–2°C (Fig. 5.2 A–B). Leaf mortality was assessed qualitatively and quantitatively, using relative conductivity (RC, an indicator of membrane integrity) and relative water content (RWC, an indicator of dessication). These indicators were negatively correlated (Pearson’s $r = −0.72$). The first frost on Oct 13 ($T_{min} = 1.7^\circ$C; $T_{leaf} = −1.2^\circ$C) did not change the appearance of the leaves, or result in substantial changes in relative conductivity or relative water content (Fig. 5.2 C–D). On Oct 26, the frost was colder ($T_{min} = 1.7^\circ$C, $T_{leaf} = −2.4^\circ$C) and water-soaked areas appeared next to leaf veins in 15–25% of leaves, but leaf punches harvested for RWC and RC showed only minor deviations from non-freezing days (Fig. 5.2 C–D). The harder frost on Nov 2–4 ($T_{min} = 0.0–0.3^\circ$C, $T_{leaf} = −2.2$ to $−3.1^\circ$C) resulted in a 5– to 6–fold increase in RC and a 35–55% reduction in RWC. This event also killed ca. 20–50% of the canopy (data not shown). On Nov 21, more than 50% of the remaining leaves were abscised after another frost event ($T_{min} = −0.8^\circ$C, $T_{leaf} = −0.9^\circ$C) (Fig. 5.2). Leaves that weren’t lost were severely damaged (wilted, water-soaked around the veins, and curled).

In 2008, leaf viability was assessed visually. Mature leaves exposed to chilling temperatures ($T_{min} = 6^\circ$C) appeared healthy (Fig. 5.3 A, D), but newly developing leaves were chlorotic (Fig. 5.4 A–B). The first frost came on Oct 30, 2008, when $T_{min} = −0.3^\circ$C (Figs. 5.3 B, E and 5.4 C–D). The canopy of some plants remained intact, while others
Figure 5.2 – Assessment of leaf mortality during fall 2006. Open symbols represent pooled measurements of plants from 5 populations (Table 5.1). Shown are (A) the mean (solid line), minimum, and maximum (dashed lines) daily air temperature at the Earth Sciences meteorological station at the University of Toronto; (B) mean maximum and minimum (solid lines) leaf temperature, logged on 2–3 leaves using 36-gauge Type T thermocouples; (C) mean (± se) relative conductivity of leaf discs (N = 8) punched from representative upper canopy leaves; and (D) mean (± se) relative water content, measured on a second set of leaf discs (N = 8) punched from the same leaves as in (C).
were more severely affected (compare Fig. 5.3 C to Fig. 5.3 F). There was no obvious difference between populations. No plant lost more than ca. 25% of its canopy (not shown). By the beginning of December, the canopy was dead after successive freezing events with $T_{\text{min}} < -2^\circ$C in November (Fig. 5.3 G). In 2009, the first damaging frost occurred after the study ended, on Nov 6 (Jul = 310) at the rooftop garden. Leaves had extensive darkened tissue around the veins (as in Fig. 5.4 C–D). The canopy was dead by the beginning of December.

In Leamington, Ontario, representative leaves were collected from eight locations in the patch on Nov 2, 2009 by Albert Tenuta. Green leaves were still present in six of the eight collections (Fig. 5.5). Minimum air temperatures at Harrow had dropped below $-1^\circ$C on Oct 11, and Oct 17, and below $-2^\circ$C ($-2.2^\circ$C) on Oct 18. Some leaves clearly survived these events. On Nov 5, after two more nights of $T_{\text{min}}$ around $-2.5^\circ$C, green leaves were still present in sheltered areas (not shown). The entire canopy was dead on Nov 14, 2009; $T_{\text{min}}$ had dropped to $-4.4^\circ$C on Nov 6 (data not shown, Fig. 5.5). On Nov 11, 2010, leaves had experienced 4 days of $T_{\text{min}} < -2^\circ$C (minimum= $-3.0^\circ$C). A few green leaves were present (not shown), but most were dead (Fig. 5.5).

**Figure 5.3 (following page)** – The effect of low fall temperatures on plant canopies in 2008. One plant from Huntington Station, New York (A–C), and one plant from Montrose, New York (D–F), represent the range of observed responses. (A, D) On Oct 4 (Jul = 278), leaves had experienced $T_{\text{min}} = 6^\circ$C but not colder, and plant canopies appeared healthy (but see Fig. 5.4 A–B). (B, E) On Oct 30 (Jul = 304), air temperature dropped below zero (to $-0.3^\circ$C) for the first time that year. Some leaves were frosted (Fig. 5.4 C–D), but the effect on the canopy was not yet apparent. (C, F) On Nov 4 (Jul = 309), air temperature had warmed to $T_{\text{min}} = 11^\circ$C, but the impact of the frost on Oct 30 was apparent in dead outer canopy leaves of some plants (F) but not others (C). (G) By Dec 2 (Jul = 337), plants had experienced 7 days of $T_{\text{min}} < -2^\circ$C (minimum = $-7.8^\circ$C); all leaves were dead, but plants regrew when transferred to the greenhouse (not shown).
Figure 5.4 – The effect of low fall temperatures on kudzu leaves in 2008. (A–B) Photographs taken on Oct 4 (Jul = 278) showing the pale color (chlorosis) of young leaves that developed in chilling but non-freezing temperatures ($T_{\min} = 6^\circ C$). (C–D) On the morning of Oct 30 (Jul = 304), air temperature had dropped below zero (to $-0.3^\circ C$) for the first time that year. Frost was most apparent on leaves that were exposed to the night sky, or where leaf movements were restricted (C). Frosted leaves developed a mosaic pattern of darkened tissue that was apparent particularly around the veins (D), and some leaves appeared completely water-soaked (compare leaves in (C) to the mature leaf in (B)). This frost event caused partial canopy death in some plants, but others were unaffected (Fig. 5.3 C, F).
Figure 5.5 – The effect of low fall temperatures on the kudzu canopy in Leamington, Ontario. On Nov 2, 2009, the canopy in Leamington had experienced 1 day of $T_{\text{min}} < -2^\circ\text{C}$. Based on representative leaf samples harvested by Albert Tenuta on Nov 2, 2009, the patch contained a full spectrum of leaf quality, from intact healthy-looking leaves to dead leaves. The mosaic pattern of necrosis around the veins in partially damaged leaves is indicative of freezing damage. On Nov 14, leaves had experienced four more days of $T_{\text{min}} < -2^\circ\text{C}$ (minimum = $-4.4^\circ\text{C}$). No living leaves remained in the canopy. On Nov 11, 2010, leaves had also experienced 4 days of $T_{\text{min}} < -2^\circ\text{C}$ (minimum = $-3.0^\circ\text{C}$). A few green leaves remained (not shown), but most leaves were dead.
In November 2009 and 2010, the condition of the kudzu canopy at the field site in Leamington, Ontario was qualitatively assessed. Moderately damaged leaves were curled, with patches of necrosis, typically around the veins or along the leaf margins. Such symptoms reduce leaf area, but the green portions of leaves typically retain photosynthetic competence (Chapter 3). Severely damaged leaves were either already fully dessicated, or appeared water-soaked, indicating a loss of compartmentation.

5.4.3 Stem elongation rate

Stem elongation rate was measured in the fall of 2008 and 2009 until growth ceased (Figs. 5.6 B, 5.7 B). Stem elongation rates were also measured in summer 2010 and used as a control (Fig. 5.8). In fall 2008 and 2009, mean growth rates did not exceed 2 cm d\(^{-1}\) (Figs. 5.6 B, 5.7 B), compared to mean growth rates of 9 cm d\(^{-1}\) in early August (Fig. 5.8 B). At the end of September, some stems were still capable of growth up to 10 cm d\(^{-1}\), half the maximum growth rate observed in August (Fig. 5.8 B). After the beginning of October, no stem exceeded 5 cm d\(^{-1}\) despite moderated temperatures (Figs. 5.6 A–B, 5.7 A–B). The slowdown in growth coincided with the first day that minimum air temperatures dropped below 8°C (Oct 2–4 (Jul = 276–278) in 2008 and Sept

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**Figure 5.6 (following page)** – Summary of growth and photosynthesis measurements during fall 2008. Open symbols represent pooled measurements of plants from 4 populations (Table 5.1). Filled diamonds are the maximum values measured on a given day. On Sept 25, Oct 6, 7, and 10 (Julian Days (Jul) = 269, 280, 281, and 284), a new leaf was selected each day (circles). Starting on Oct 12 (Jul = 286), two matched leaves per plant were followed for the remainder of the study (triangles). Shown are (A) mean (solid line), minimum and maximum (dashed lines) daily air temperature at the Earth Sciences meteorological station; (B) mean (± se) stem elongation rates; (C) mean (± se) net CO\(_2\) assimilation rates; (D) mean (± se) stomatal conductance; and (E) mean (± se) \(C_t/C_a\).
30 (Jul = 273) in 2009). In 2008, when air temperature warmed again, stem elongation rates failed to respond (Fig. 5.6 A–B).

Stem mortality rates were also assessed in 2008. Prior to Oct 4, 2008 (Jul = 277), no (non-accidental) stem death was recorded (Table 5.3). Between Oct 4 and 10 (Jul = 278–284), < 10% of stems were dead on each day. This period of low mortality was characterized by mean minimum and maximum temperatures of 8.7°C and 16.8°C (absolute $T_{\text{min}}$ = 6.6°C) (Fig. 5.6). Mortality rates increased to 10–20% between Oct 10 and 14 (Jul = 284–288), even though $T_{\text{min}}$ did not go below 9°C. On Oct 17, 2008 (Jul = 291), air temperatures dropped to 9.4 (5.9, 13.6)°C; one day later all stem tips had stopped growing and half had died. By Nov 5, all remaining shoot tips were dead (Table 5.3, Fig. 5.6). There were small difference between populations in both 2008 and 2009, but no indication of a latitudinal gradient in the sensitivity of stem elongation rates to fall chilling.

To evaluate the temperature response of growth, average growth measurements for the two years of fall experiments (Figs. 5.6 B, 5.7 B) and the year of summer measurements (Fig. 5.8) were fit to mean $T_{\text{max}}$ during the time period (1–10 days) between measurements (Fig. 5.9). Mean $T_{\text{max}}$ explains 52% of the variance in all of the data, but only 6% of the fall rates, indicating that stems in the fall loose their ability to respond to warm days. The upper and lower quintiles show the same pattern (Fig. E.1).

Figure 5.7 (following page) – Summary of growth and photosynthesis measurements during fall 2009. Open symbols represent pooled measurements of plants from 3 populations (Table 5.1). Filled diamonds are the maximum values measured on a given day. Shown are (A) mean (solid line), minimum and maximum (dashed lines) daily air temperature at the Earth Sciences meteorological station; (B) mean (± se) stem elongation rates; (C) mean (± se) net CO$_2$ assimilation rates; (D) mean (± se) stomatal conductance; and (E) mean (± se) $C_l/C_a$. 
Figure 5.8 – Summary of growth and photosynthesis measurements during summer 2010. Open symbols represent pooled measurements of plants from 9 populations (Table 5.1). Filled diamonds are the maximum values measured on a given day. Shown are (A) mean (solid line), minimum and maximum (dashed lines) daily air temperature at the Earth Sciences Centre meteorological station; and (B) mean (± se) stem elongation rates.
**Table 5.3** – Stem mortality assessed on stems used for growth measurements in fall 2008. Values are the % of total stems that were dead on each day. The total number of stems was 36–38 on all days except Oct 14 ($N = 31$), Oct 18 ($N = 28$) and Nov 5 ($N = 14$), where dead stems were not replaced because there were no more living stems.

<table>
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<th>Date in 2008 (Jul)</th>
<th>% Stem mortality</th>
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</thead>
<tbody>
<tr>
<td>26 Sep–3 Oct (270–277)</td>
<td>0</td>
</tr>
<tr>
<td>4 Oct (278)</td>
<td>5</td>
</tr>
<tr>
<td>5 Oct (279)</td>
<td>3</td>
</tr>
<tr>
<td>6 Oct (280)</td>
<td>5</td>
</tr>
<tr>
<td>7 Oct (281)</td>
<td>3</td>
</tr>
<tr>
<td>8 Oct (282)</td>
<td>0</td>
</tr>
<tr>
<td>9 Oct (283)</td>
<td>3</td>
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Figure 5.9 – Stem elongation rate as a function of mean maximum temperature over the 1–11 d period pertaining to the measurement. Open symbols are the overall means, growth quintiles are shown in E.1. Growth rates on each day of measurement on the roof of the Earth Sciences Centre in summer 2010 (circles), fall 2008 (diamonds), and fall 2009 (squares) (Figs. 5.6, 5.7, 5.8). Regression lines are fit to all three datasets simultaneously. The regression for the fall data is \( y = 0.11x - 1.4 \) (adj. \( R^2 = 0.06 \)). The line fit to summer and fall mean growth rates is \( y = 0.52x - 8.6 \) (adj. \( R^2 = 0.52 \)).
5.4.4 Gas exchange and chlorophyll fluorescence

Kudzu leaves retained their ability to achieve high photosynthesis rates until growth stopped completely ($> 15 \text{ µmol}^{-1} \text{ m}^{-2} \text{ s}^{-1}$, Figs. 5.6 B–C, 5.7 B–C), after which photosynthesis rates to low but non-zero rates. This decline in $A_{\text{net}}$ was also associated with a steady decline in minimum temperatures from 10–13°C to 1–2°C for the first time since the summer. Leaves do not appear to recover from this decline; in 2009, leaf photosynthesis was still $< 5 \text{ µmol}^{-1} \text{ m}^{-2} \text{ s}^{-1}$ after two weeks of warmer temperatures. The decline in $A_{\text{net}}$ was associated with declines in stomatal conductance and an increase in $C_i$ once mean $A_{\text{net}}$ fell below 5 µmol$^{-1}$ m$^{-2}$ s$^{-1}$ (Figs. 5.6 C–E, 5.7 C–E).

Frost kills part of the canopy, but leaves that survive retain some photosynthetic competence. On Oct 30, 2008 (Jul = 304), when $T_{\text{min}} = -0.3^\circ\text{C}$, 8% of leaves had $A_{\text{net}} > 2 \text{ µmol}^{-1} \text{ m}^{-2} \text{ s}^{-1}$. The next day, 60% (14 of 24) of the measurement leaves had died, but

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Figure 5.10 (following page) – Summary of air temperature and fluorescence parameters measured during fall 2008. Symbols represent pooled measurements of plants from 4 populations (Table 5.1). On Sept 25, Oct 6, 7, and 10 (Julian Days (Jul) = 269, 280, 281, and 284), a new leaf was selected each day (circles). Starting on Oct 12 (Jul = 286), two matched leaves per plant were followed for the remainder of the study (triangles). Shown are (A) mean (solid line), minimum and maximum (dashed lines) daily air temperature at the Earth Sciences meteorological station; (B) the mean $(\pm \text{ se})$ maximum efficiency of PSII measured before dawn (predawn $F_v/F_m$); (C) the quantum yield of photochemical energy dissipation ($\Phi_P$); (D) the mean $(\pm \text{ se})$ quantum yield of non-photochemical energy dissipation $(1-\Phi_P)$ partitioned into regulated dark-reversible dissipation ($\Phi_{\text{Reg}}$), combined constitutive and fluorescence dissipation ($\Phi_{f,\text{CON}}$) and dissipation related to the inactivation of PSII ($\Phi_{\text{NF}}$) such that $\Phi_P + \Phi_{\text{Reg}} + \Phi_{f,\text{CON}} + \Phi_{\text{NF}} = 1$; and (E) the mean $(\pm \text{ se})$ ratio of $\Phi_{\text{Reg}}$ to $\Phi_{\text{NF}}$ (crosses) emphasizing the shift from regulated dissipation to photo-inactivation during cold events.
Figure 5.11 – Summary of air temperature and fluorescence parameters measured during fall 2009. Open symbols represent pooled measurements of plants from 3 populations (Table 5.1). Shown are (A) the mean (solid line), minimum and maximum (dashed lines) daily air temperature at the Earth Sciences meteorological station; (B) the mean (± se) maximum efficiency of PSII measured before dawn (predawn $F_v/F_m$); (C) the quantum yield of photochemical energy dissipation ($\Phi_P$).
two of the 10 survivors still had $A_{\text{net}}$ of 4–5 $\mu$mol$^{-1}$ m$^{-2}$ s$^{-1}$ (Fig. 5.6 C). Predawn $F_v/F_m$ remained relatively constant, but $\Phi_P$ declined with $A_{\text{net}}$ (Fig. 5.10 B–C, 5.6 C). Analysis of fluorescence partitioning in the first half of 2008, shows that $\Phi_{\text{NF}}$, which represents light-induced deactivation of PSII, increased relative to regulated non-photochemical dissipation ($\Phi_{\text{Reg}}$) when $T_{\text{min}}$ went below $\sim 10^\circ C$ (Fig. 5.10 A, E). In 2009, 25% of leaves (5 of 20) that were on the vines on Nov 1 (Jul = 305) had $A_{\text{net}} > 2$ $\mu$mol$^{-1}$ m$^{-2}$ s$^{-1}$.

Predawn $F_v/F_m$ declined more than in 2008 when temperatures approached 0$^\circ C$, but it recovered once temperatures warmed, while $\Phi_P$ did not recover (Fig. 5.11). In both years, the best leaves on Jul = 305 were from both New York and Mississippi, so there was no clear relationship between geographic origin and photosynthetic competence.

The relationship between $A_{\text{net}}$ and climate could not be evaluated because $A_{\text{net}}$ was measured at in situ leaf temperature (which covaries with air temperature variables) rather than at a constant temperature. To evaluate whether the temperature response of photosynthesis in the fall deviates from that in the summer, $A_{\text{net}}$ was plotted against measurement temperature (in an $A$–$T$ curve) and compared to $A$–$T$ curves measured on potted plants in the summer (Fig. 4.5 E). Fall data correspond to the summer data at measurement temperatures $\geq 22^\circ C$. At measurement temperatures less than 22$^\circ C$, fall $A_{\text{net}}$ diverges from the expected $A_{\text{net}}$ of leaves that are warm or cool-acclimated but grown in otherwise optimal conditions. Fall $A_{\text{net}}$ is 50% of that predicted by the summer $A$–$T$ curves at 15$^\circ C$. At measurement temperatures below 13$^\circ C$, $A_{\text{net}}$ is nil rather than 6 $\mu$mol$^{-1}$ m$^{-2}$ s$^{-1}$ (Fig. 5.12). There was no relationship with minimum morning temperature (results not shown).

### 5.4.5 Climate analysis

To evaluate when the growing season ends in more northern locations in Ontario where kudzu is not present, the date that temperatures first cross thresholds associated with declines in growth and photosynthesis were calculated, as well as the first date when
Figure 5.12 – Mean (± se) net CO_2 assimilation rates (from Figs. 5.6 C and 5.7 C) plotted against measurement (leaf) temperature. Leaf temperature in the gas exchange cuvette was set to match in situ leaf temperature. N ≥ 7 leaves at all temperatures except 12°C (N = 5), and 11°C and 27°C, where N = 1. Loess (local) regression fits to summer \( A_{\text{net}} \) vs. measurement temperature curves measured on potted plants grown at 22°/14°C and 31°/22°C day/night temperatures (from Fig. 4.5 E) are shown as solid and dashed lines, respectively.
extensive leaf mortality would occur (Table 5.4). At Guelph, Barrie, and North Bay, temperatures cross the threshold associated with slowed growth \((T_{\text{min}} < 8 \degree C)\) 1–2 weeks earlier on average than in Leamington (Table 5.4 A). In cold years these temperatures can occur as early as the first week of September, a little more than one week earlier than the earliest date observed in Leamington. The latest dates are more similar, falling at the end of September or in early October at all stations.

The threshold associated with a decline in photosynthesis rate \((T_{\text{min}} < 5 \degree C)\) is first crossed in mid-September in Guelph, Barrie, and North Bay, compared to early October in Leamington (Table 5.4 B). However, the time period between the mean date of growth decline and the mean date of \(A_{\text{net}}\) decline is similarly short (6–10 days) in all four locations (Table 5.4 A–B). In cold years, this period is reduced to 3–8 days at all four stations. The first frost, which should destroy part (but not all) of the canopy, comes 12 days earlier in Guelph than in Leamington, 14 days earlier in Barrie, and 19 days earlier in North Bay (Table 5.4 C). The earliest date for the first hard frost \((< -2 \degree C)\) is Oct 6 at the three northern stations, which is 12 days earlier than the earliest date in Leamington. The latest first frost in the last 9 years was in mid-November, except in North Bay, when the first hard frost came at the end of October (Table 5.4 C). Once photosynthesis has declined, the canopy would have about one month of modest photosynthesis rates at all locations before a hard frost (Table 5.4 B–C).

### 5.5 Discussion

The goal of this study was to evaluate the impact of fall frost on kudzu growth, photosynthesis, and canopy drop. The results show that kudzu is more frost tolerant than the literature implies (Winberry and Jones, 1973; Miller and Edwards, 1983; Shurtleff and Aoyagi, 1985). Kudzu can tolerate mild frosts \((T_{\text{leaf}} \text{ between } 0 \degree C \text{ and } -2 \degree C)\). It looses leaves in harder frosts, but multiple hard frosts \((T_{\text{leaf}} \leq -2 \degree C)\) are required to
Table 5.4 – The first date (and Julian day) in Sep–Dec that minimum temperatures cross thresholds corresponding to (A) the irreversible decline in maximum growth rates to \(< 2 \text{ cm d}^{-1}\) (from Figs. 5.6 B, 5.7 B); (B) the irreversible decline in $A_{\text{net}}$ below 5 $\mu\text{mol}^{-1} \text{ m}^{-2} \text{ s}^{-1}$ (from Figs. 5.6 C, 5.7 C); and (C) extensive leaf death (Figs. 5.2, 5.5).

Climate data for the 9 year period 2002–2010 were obtained from Environment Canada (www.climate.weatheroffice.gc.ca). Station information is given in the text.

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completely kill the canopy (Figs. 5.3, 5.5). Stems were sensitive to temperature declines, losing their capacity for rapid growth once $T_{\text{min}}$ dropped below $8^\circ C$ (Figs. 5.6, 5.9), but photosynthesis was not as sensitive to chilling. $A_{\text{net}}$ stayed high until $T_{\text{min}}$ dropped below $5^\circ C$ (Figs. 5.6, 5.7). Subsequently, some leaves maintained modest photosynthesis rates ($A_{\text{net}} = 2-5 \mu\text{mol}^{-1} \text{m}^{-2} \text{s}^{-1}$) and membrane integrity until they were eventually killed by successive hard frosts in November or December (Fig. 5.6).

5.5.1 Implications for kudzu’s northern range limit

To evaluate whether fall frosts could prevent kudzu success north of its current range limit, climate data from the last 9 years (2002–2010) was analyzed for Leamington and three more northern locations in Ontario where kudzu is not yet present: Guelph, Barrie and North Bay. The first day of minimum temperatures below $8^\circ C$, which is when stem elongation was reduced to less than $2 \text{ cm d}^{-1}$ in this study, arrives 1–2 weeks earlier in the three northern locations than in Leamington (Table 5.4 A). The data show that growth responds to these low temperature thresholds, so stem elongation should stop 1–2 weeks earlier (Figs. 5.6 A–B, 5.7 A–B).

Temperatures cool enough to stop stem elongation can occur in August north of Leamington (results not shown), but kudzu can recover from chilling in the summer. A growth chamber experiment that treated kudzu plants grown at $28^\circ /20^\circ C$ (14 hour days) to two days at $10^\circ /5^\circ C$ or $15^\circ /5^\circ C$ showed that growth rebounded within three days to pre-chill rates once plants were transferred back to $28^\circ /20^\circ C$ (Appendix A). On August 1, days are longer than 14 hours at all four stations (14:49 hours in North Bay vs. 14:27 hours in Leamington), while on Oct 1 (when growth slowed in this study), they are equal, and three hours shorter (11:45 hours vs. 11:42 hours). Long days may promote growth and alter how kudzu responds to low temperature cues. Grapevines maintained in a long day treatment had higher growth rates and less cold tolerance than plants subjected to shorter days (Fennell and Hoover, 1991). Low temperature cues also failed to induce cold
hardening in conifer seedlings if days were still long (Christersson, 1978). The evidence indicates that kudzu should be able to recover from cold events that occur in the summer months.

The growing season should be 1–2 weeks shorter in Barrie and North Bay than in Leamington because temperature thresholds that stop growth and photosynthesis occur 1–2 weeks earlier. This matters less than the equivalent loss of time in spring because spring is sunnier. In Leamington, Ontario, solar insolation peaks at 22–23 MJ/m² in June, dropping to less than half that in October (9–10 MJ/m²) (Natural Resources Canada, 1984). In North Bay, solar insolation is similar to that in Leamington in June (21–22 MJ/m²), but it drops off a little more rapidly in October (down to 7–8 MJ/m²) (Natural Resources Canada, 1984). Spring days are also up to 3 hours longer, so one week lost in May or June, due e.g., to a severe chill, would prevent kudzu from using the long sunny days to assimilate carbon and grow (Chapter 3). This would have a greater impact on kudzu’s annual carbon balance than one less week of growth or photosynthesis in autumn.

The combination of declining solar insolation, reductions in leaf area due to frost, and the inability to achieve high photosynthesis rates mean that fall carbon gain in kudzu is probably low, but it may not be inconsequential. In contrast to most temperate deciduous plants, which induce leaf senescence in the fall prior to killing temperatures, kudzu maintains a canopy until low temperature kill the leaves. This trait should help kudzu increase its total carbon gain. Kudzu photosynthesis rates in the fall may seem low, but they are comparable to other temperate vines that retain their leaves in the cold season. English ivy (Hedera helix) leaf photosynthesis drops from 10 \( \mu \text{mol}^{-1} \text{m}^{-2} \text{s}^{-1} \) to below 5 \( \mu \text{mol}^{-1} \text{m}^{-2} \text{s}^{-1} \) when minimum air temperatures drop below zero (Oberhuber and Bauer, 1991). Overwintering leaves of the invasive vine Lonicera japonica and its native congener L. sempervirens average \( \sim 3 \mu \text{mol}^{-1} \text{m}^{-2} \text{s}^{-1} \) in January (Schierenbeck and Marshall, 1993), but L. japonica retains more leaves longer into the cold season. This
results in about $5 \times$ more total carbon gain in January, which is a small proportion of the annual carbon budget, but it is nevertheless interpreted to contribute to the invasive’s success relative to the native (Schierenbeck and Marshall, 1993). Longer retention of the leaf canopy is also interpreted to contribute to greater invasive potential in *Phalaris arundinacea*, which maintains a leaf canopy longer than the native sedge *Carex stricta*, and therefore has greater seasonal carbon gain (He et al., 2011).

Longer leaf retention is therefore likely to increase kudzu’s invasive potential in the temperate zone. Kudzu seedlings grown in a glasshouse in Kobe, Japan ($34.7^\circ$N, $135.2^\circ$E) continued to increase stem and root dry matter (and girth) for three months after main stem elongation ceased (Tsugawa et al., 1979). Early spring growth depends on stored carbon, as does kudzu’s ability to recover from canopy damage (Bailey, 1939). In grapevine, such shifts in assimilate allocation from elongation to storage in the fall are considered to be critical for its success (Mooney and Gartner, 1991). If the time period between the end of stem elongation and leaf kill were substantially shorter north of Leamington, this could compromise kudzu’s ability to establish there, but it is not. In Guelph and Barrie, kudzu has an average of 40 days and 43 days, respectively, to build upon its carbohydrate stores, compared to 44 days in Leamington (Table 5.4).

The results of this study show that there is no strong evidence that fall frost alone explains kudzu’s northern range limit. Based on these results, kudzu should be able to establish as far north as Barrie, and possibly North Bay. This study also shows that kudzu is well-positioned to take advantage of warmer fall temperatures and later fall frosts associated with global warming. Southern and central Ontario have already experienced $3^\circ$C warming of extreme minimum autumn temperatures since 1900, and a 10–20 day later onset of fall frost (Bonsal et al., 2001). These trends are expected to continue; unless emissions are reduced, the first autumn frost should come 16 days later by 2064 (Hayhoe et al., 2006) and temperatures are expected to warm in early fall when solar insolation is highest (Bonsal et al., 2001). By retaining leaves, rather than
senescing them, kudzu should be able to exploit years with later fall frosts and increase its carbon stocks, which would increase its resistance to episodic spring frosts (Chapter 3; Bailey, 1939). Conservation managers cannot depend on fall frost to prevent kudzu from establishing in areas beyond kudzu’s northern range limit, especially in the context of global warming.
Chapter 6

Conclusion

This thesis evaluated the hypothesis that low temperatures determine the northern range limit of kudzu in North America. This hypothesis was motivated by a close biogeographic correlation between kudzu’s northern range limit, and an isocline representing $-20^\circ$C minimum winter temperature (Sasek and Strain, 1990). This correlation was supported by anecdotal evidence that kudzu was intolerant of cold temperatures, but quantitative studies that identified a physiological mechanism were lacking. My first objective was to evaluate whether kudzu’s distribution has tracked the northward movement of minimum winter temperatures. I then focused on how low temperatures could prevent kudzu establishment throughout the year. Specifically, I hypothesized that (1) minimum winter temperatures prevent winter survival; (2) that spring chilling temperatures prevent timely development of the canopy and photosynthetic competence; (3) that cool summer temperatures prevent the achievement of peak growth and photosynthesis rates; and (4) that autumn frost prevents the orderly onset of autumn dormancy. In the remainder of this chapter, I will briefly review the main results of my thesis and discuss their broader implications.
6.1 Summary of results

6.1.1 Is kudzu’s northern range limit correlated with minimum winter temperature?

The first objective of my thesis was to validate that kudzu’s northern range limit in North America has migrated northward along with the minimum winter isocline corresponding to $-20^\circ$C. In Chapter 2, I evaluated kudzu occurrence in all of the states in the northeastern U.S., visiting and vouchering most of the peripheral populations. If $-20^\circ$C minimum winter temperature is preventing kudzu establishment, then a low frequency of days (0–2 days per year) that meet or exceed that threshold should correlate with the northern range limit currently, and in the past. In collaboration with Katharine Hayhoe, I confirmed that the northern range limit of kudzu’s distribution has moved northward between 1970 and 2009, together with the the isocline representing a low frequency of days at $-20^\circ$C or below. These results strongly suggest that kudzu is limited by minimum winter temperature. We then modeled the future potential distribution of kudzu, showing that kudzu should be able to survive winter in most of the northeastern U.S. and southwestern Ontario by 2069. The validity of these model projections depends on whether low temperature does in fact limit kudzu’s northward range expansion.

6.1.2 Winter freezing and kudzu survival

The most obvious way that low temperature could limit kudzu’s range expansion is by freezing mortality. Thus, the second objective of my thesis was to test the hypothesis that kudzu cannot survive temperatures below $-20^\circ$C (Chapter 2). I first determined the amount of tissue damage kudzu stems can sustain and still survive. Differences in freezing tolerance between stems from northern and southern populations, and between above- and below-ground stems suggested that kudzu may be able to deepen its freezing tolerance if exposed (acclimated) to colder temperatures. I tested for acclimation by sampling
above- and below-ground stems collected throughout the winter at a northern population in Leamington, Ontario. The results showed that both above- and below-ground tissues acclimated to cold temperatures, and that both types of stem could survive low winter temperatures that occur beyond kudzu’s northern range limit. Most importantly, below-ground stems, which are protected from air temperature extremes by soil, can tolerate soil temperatures that occur up to 400 km north of the current range limit. There was no strong evidence that freezing temperatures were preventing kudzu establishment in areas immediately to the north of kudzu’s range limit. This unexpected result generated two alternative hypotheses: that a correlate of minimum winter temperature was instead preventing establishment, or that kudzu was not limited by temperature. In the remainder of my thesis, I test the first possibility.

6.1.3 Spring chilling and kudzu canopy development

The third objective of my thesis was to evaluate the hypothesis that spring chilling prevents the timely development of the kudzu canopy (Chapter 3). If spring chill is limiting, I would expect a severe chill to result in a substantial delay of canopy development, extensive mortality, or an impaired ability to recover from the event. I measured growth rates and photosynthesis rates during two springs in a population in Leamington, Ontario, located at the northern limit of kudzu’s naturalized range. In the first year, an early budbreak was followed by a severe chill. This event halted growth and photosynthesis in existing stems and leaves, caused some mortality, and impaired photosynthesis in leaves that were still immature when chilled. Nevertheless, once temperatures warmed, there were no detectable delays in growth recovery due to the event, and mature leaves that were chilled regained the ability to achieve high photosynthesis rates once mornings warmed. By the beginning of June, less than three weeks following the chill, the canopy had closed and both growth and photosynthesis were similar to summer levels. The population went on to produce viable seed and survive the winter. The following year,
budbreak was later, and the patch experienced a more moderate chill, but there were no lasting effects. I compared the frequency of chills in Leamington to that of locations further north. Spring chills are more common north of kudzu’s current range limit, but given that kudzu appeared to recover fully in Leamington, a single event, even if it happens every year, is unlikely to prevent kudzu establishment. Successive chills (or frosts) might, but those start to occur several hundred km north of kudzu’s range limit. Thus, there is no strong evidence that spring chills are impairing kudzu’s ability to colonize areas north of its current range limit.

6.1.4 Cool summer temperatures and kudzu performance

My fourth objective was to evaluate the hypothesis that cool summer temperatures prevent kudzu from achieving peak growth and photosynthesis rates (Chapter 4). If so, I would expect to see evidence of sustained slow growth or lasting reductions in photosynthesis rates, due e.g., to cool nights. Using a combination of field experiments at Leamington, Ontario, and controlled temperature studies, I show that stem growth responds rapidly to maximum, rather than minimum, temperature. Growth stops around 10°C, but most growth occurs during the day, when temperatures are much warmer. Moreover, cool nights typical of kudzu’s northern range limit do not prevent high growth rates, so there is little evidence that growth is impaired by cool temperature in the summer. Leaves cannot boost photosynthesis at cool growth temperatures, but leaf temperatures are usually within the optimum range. Kudzu’s high leaf area index and fast growth rates, combined with a sustained capacity for moderate $A_{\text{net}}$, mean that kudzu’s net canopy-level carbon gain is likely to remain high. Warm-acclimated stems can recover from exposure to 10°C/5°C day/night so an atypical cold spell in summer is also not likely to be limiting. In areas north of kudzu’s current range limit, summer temperatures are somewhat less favourable for maximum growth and photosynthesis rates, but major differences do not occur for 400 km north of the range limit. Thus, there is no strong
evidence that cool summer temperatures are impairing kudzu’s ability to colonize areas north of its current range limit.

6.1.5 Autumn frost and kudzu dormancy

My fifth objective was to evaluate the hypothesis that autumn frost caused premature leaf mortality and prevented the onset of autumn dormancy (Chapter 5). Deciduous plants that are adapted to temperate climates often direct photosynthate away from growth and towards storage in preparation for winter (Chapin et al., 1990). This hypothesis would therefore be supported if an autumn frost caused high leaf mortality while growth rates were still high. Using potted kudzu plants from a range of populations growing in a rooftop garden, I assessed leaf mortality, growth, and photosynthesis over three years. Growth rates decline irreversibly in early autumn, well before the first frost, but leaves persist and maintain modest photosynthesis rates until they are killed by successive frosts. By retaining photosynthetically competent leaves, rather than senescing them, kudzu can exploit warm periods in autumn and potentially increase stores of carbohydrate. Autumn senescence would be advanced 1–2 weeks at locations several hundred kilometers north of kudzu’s range limit, but this reduction in the growing season should not matter because solar insolation is also low. At areas more immediately north of kudzu’s current range limit, the differences are smaller. Thus, there is also no strong evidence that autumn frosts are preventing kudzu from migrating northward.

6.1.6 Alternative hypotheses and opportunities for future work

The hypothesis that low temperatures control the northern range limit of kudzu in North America is not supported by the results of my thesis. There is no obvious physiological mechanism in any one season that can account for the correlation between low temperature and kudzu’s northern range limit. If low temperatures are not limiting kudzu’s northern range limit, as my data indicates, then what is? In this section, I will consider
two alternative hypotheses: that kudzu is temperature limited, but in a way my study could not detect, or that kudzu is limited instead by dispersal.

I have presented strong evidence that kudzu can establish new populations north of its current range limit. This is important because isolated populations, such as the kudzu population in Leamington, can foreshadow a severe invasion. A detailed study of the migration of another invasive species, *Bromus tectorum*, shows that it existed for 30 years as small, isolated populations in the Intermountain West before population density exploded in the next 10 years (Mack, 1986). Similar patterns of invasion have been observed in *Picea abies* invasion during the Mid-Holocene. Radio-carbon dating of partially fossilized wood remnants shows that, in one location in Sweden, *P. abies* existed more than 2000 years earlier than pollen records suggest (Kullman, 1995). Outlier populations are difficult to detect in pollen records because they do not produce enough pollen (Davis et al., 1991), so this find suggests that *P. abies* established first as isolated populations. A model of migration that starts with a few isolated populations far from the main body of the distribution may also explain why observed migration rates (e.g., in the Holocene) have been much faster than expected based on mean dispersal rates (Clark, 1998; Pulliam, 2000; Kot et al., 1996). Metapopulation theory also supports the notion that migration requires a critical mass of isolated populations (Jongejans et al., 2008; Parmesan, 1999; Pulliam, 2000). In the case of invasive species, isolated populations at the range periphery, such as the Leamington kudzu populations, should be seen as an opportunity to prevent future spread.

Nevertheless, an important next step would be to evaluate experimentally whether kudzu persistence would be prevented by the cumulative effects of many small physiological limitations. This might happen in the event of one or more rare extreme episodes, such as partial root kill in winter, followed by successive chills in the spring or a severe cold front in the summer. Kudzu would then be required to regrow a second canopy before its carbohydrate stores are fully replenished, which would reduce biomass and
viability the following year (Bailey, 1939; Terrill et al., 2003). The cost of canopy re-
growth would be exacerbated if summers were also cooler and shorter (Chapters 4, 5).
Weakened plants would also have reduced competitive ability as well as reduced ability
to resist infection and herbivory. If kudzu experienced two or more successive years with
these conditions, then it may not be able to persist.

The best way to evaluate factors preventing persistence would be to plant kudzu
beyond its current distribution limit and evaluate population viability. When cultivated
and fertilized, kudzu populations take 3–4 years to establish full productivity (Bailey,
1939); naturalized populations should take at least that long, so this would be a multi-
year study. The risk of kudzu escape precluded the inclusion of such an experiment
in this thesis. Based on the performance of the peripheral population in Leamington,
I would nevertheless expect that kudzu has the potential to persist, and be invasive,
in southern Ontario in the current climate. The Leamington population has thrived
in that location for at least seven years. It achieves growth and photosynthesis rates
that are similar to those observed in more southern locations where kudzu is a problem
(Wechsler, 1977; Forseth and Innis, 2004; Forseth and Teramura, 1987). In Leamington,
kudzu vines invade the neighbouring cultivated field to a distance of ca. 10 m, requiring
active management to prevent kudzu from overtopping the crop (H.A. Coiner, personal
observation). Kudzu clearly has the capacity to be invasive in other areas with climates
similar to Leamington, such as the Niagara Peninsula. In colder areas, such as the Guelph
agricultural region ca. 250 km north-east of Leamington, kudzu may still be able to be
invasive.

A second alternative hypothesis, and the one I think is most likely, is that kudzu is
limited by dispersal (Chapter 2). There is ample evidence that, in the past, the establish-
ment of kudzu populations has been human-mediated. Millions of kudzu seedlings were
planted by the U.S. government in the height of kudzu’s popularity (Winberry and Jones,
1973; Forseth and Innis, 2004; Blaustein, 2001). More recently, new kudzu populations
tend to be located in disturbed and high-traffic areas, or on embankments, which is also suggestive of human-mediated dispersal, intentional or otherwise (McClain et al., 2006). There is also little evidence that kudzu disperses to new locations on its own. The reasons for this are puzzling. Kudzu sets viable seed (Robert Nurse, personal observation; McClain et al., 2006), and the prodigious use of seedlings by the U.S. Department of Agriculture demonstrate that seedlings can establish viable populations, albeit with cultivation (Winberry and Jones, 1973; Forseth and Innis, 2004; Blaustein, 2001). Seedlings can germinate in a range of soil types and temperature regimes (Robert Nurse, unpublished data; Susko and Mueller, 1999). Nevertheless, seedlings are rarely observed, and when they are, survivorship is low (Forseth and Innis, 2004; Abramovitz, 1983). Seeds need scarification and tend not to disperse far on their own (Abramovitz, 1983; Susko et al., 2001). Still, large mammals browse kudzu, so ingested seeds could conceivably be both scarified and transported, with fertilizer, to a new location (H.A. Coiner, personal observation). The circumstantial evidence suggests that there is a barrier to recruitment by seed, and no known natural dispersal mechanism. If so, and if humans are the sole (or most important) dispersers, then this should impact the types of management and policy measures that are implemented in non-invaded areas. Experimental evaluation of kudzu dispersal and recruitment is greatly needed.

6.2 Implications for species distribution modeling and conservation

In Chapter 2, I present a model of kudzu’s future distribution that is based on the assumption that there is a mechanistic relationship between minimum winter temperature and kudzu’s northern range limit. The results of this thesis do not support this assumption. Instead, they indicate that kudzu currently has the ability to invade well into southern Ontario. This means that the model projections underestimate kudzu’s invasive
potential. Given that kudzu is already present in Ontario, it should be a priority species for early detection programs and regulatory legislation in southern Ontario.

The assumption that species distributions are temperature limited is not restricted to kudzu; it is at the core of most models of species distributions, but what is the evidence that it is correct? At the continental scale, vegetation patterns are well-defined by climatic patterns (von Humboldt and Bonpland, 1807; Shreve, 1914; Schimper and von Faber, 1935; Hardy, 1925; Polunin, 1960; Woodward and Williams, 1987). Vegetation patterns have also tracked past climatic changes (Williams et al., 2004; Jackson and Overpeck, 2000). At the scale of the individual or population, plants have strong and well-characterized responses to temperature. Temperature influences most aspects of plant physiology and life history, including photosynthesis (Berry and Raison, 1981), respiration (Atkin and Tjoelker, 2003), growth (Atkin et al., 2006), phenology (Kramer, 1995), cold acclimation (Ensminger et al., 2006), sexual reproduction (King, 2011), recruitment (Ball et al., 1997), and survival (Inouye, 2000). There is also ample evidence that the distributions of individual species are shifting in response to recent climate change (Kelly and Goulden, 2008; Parmesan and Yohe, 2003; Walther et al., 2002, 2005; Root et al., 2003). In the face of these well-supported observations, the conclusion that temperature limits species distributions seems self-evident. Yet, the body of empirical evidence that supports this conclusion is small compared to the literature at the individual and continental scales, and it is much more equivocal.

The most convincing studies that support the hypothesis that temperature limits species distributions show that there is a barrier to population establishment or persistence beyond the range limit. For example, cold air drainage into valleys was found to cause high seedling mortality in *Euterpe edulis* seedlings (a tree palm), which disperses readily into the valleys where adults are absent (Gatti et al., 2008). Establishment of snow gum seedlings at their poleward range limit occurs in sites protected from the damaging combination of night-time radiation frost and high light in winter (Ball et al., 1991).
Similarly, seedlings of the early-successional *Pinus albicaulis* were able to establish and survive in exposed microsites at tree-line while seedlings of *Picea engelmannii*, a late-successional spruce, could not (Bansal and Germino, 2010). This difference in survival was linked to the ability of pine but not spruce seedlings to maintain favourable carbon balance in chilling conditions typical of the microsites (Bansal and Germino, 2010).

In other studies, where dispersal has been facilitated with transplant experiments, little to no loss of fecundity or viability was found beyond the range limit. One of the most thorough studies of this type was conducted 25 y ago on *Lactuca serriola*, a weedy but native annual in Britain. In a survey of 36 marginal populations, there was no morphological evidence that species “vigour” declined at its northern range limit (Carter and Prince, 1985). When populations were seeded beyond the range to the north and to the west, seeds germinated and developed into thriving plants that had morphology and fecundity similar to plants within the natural range (Prince and Carter, 1985). Moreover, populations self-seeded for several years following the study, indicating that they could also persist beyond the range limit (Prince and Carter, 1985). Similarly, populations of a non-indigenous species (*Heracleum mantegazzianum*) that were established beyond its range limit in Britain did not show clear differences in survival or other life history characters when compared to populations in its current range (Willis and Hulme, 2002). Reciprocal transplants of four *Protea* species along an environmental gradient in South Africa found that abiotic limitations did not explain the observed distribution limits (Latimer et al., 2009). *Oxalis pes-caprae*, an introduced weed in Crete, was transplanted beyond its elevational limit; plants survived and produced bulbils (asexual propagules) large enough for population persistence at elevations 150 m higher than the range limit, indicating that climate was also not limiting migration in this case (Ross et al., 2008).

In another group of studies in which physiological temperature responses are compared to conditions at the range limit, the hypothesis that temperature limits range distributions is supported in some cases. For example, the northern range limits of four
columnar cacti species in southwestern U.S. and Mexico are associated with increased apical damage caused by chilling temperatures in winter (Nobel, 1980a,c,b). *Larrea tridentata* xylem cavitates when exposed to freezing temperatures typical of its northern range limit in the southwestern U.S. (Pockman and Sperry, 1997). Chilling temperatures also help maintain mangrove forest structure and biodiversity in addition to contributing to mangrove global range limits (Duke et al., 1998; Ross et al., 2009; Kao et al., 2004). In other cases, as with my study, no obvious physiological mechanisms were found. For example, a study that grew eight species of temperate and tropical trees in four temperature regimes found no conclusive evidence that temperate and tropical trees were mutually excluded from warm and cool temperature regimes, respectively (Cunningham and Read, 2003). Instead, all species grew more at warm temperatures (Cunningham and Read, 2003). In a comparison of freezing tolerance and distribution of 70 tree species, patterns of hardiness broadly matched climate zones for some species. Others, including *Magnolia grandiflora*, *Liquidambar styraciflua*, and *Ulmus americana*, were more freezing resistant than suggested by their natural distribution, and artificial populations had already been established outside the range, corroborating the evidence that freezing was not limiting (Sakai and Weiser, 1973). Two other species, *Populus deltoides* and *Salix nigra*, could tolerate much colder temperatures than those experienced in their native ranges (Sakai and Weiser, 1973).

Taken together, the evidence shows that despite large-scale correlations between vegetation and climate, and individual temperature responses, it is not at all self-evident that temperature limits species distributions. Even in cases where evidence is present, temperature often accounts for just a small portion of the range boundary. A recent review of differences between range centers and margins had similar findings. Sexton and co-authors found that evidence for decreased abundance and fitness at range limits is lacking (Sexton et al., 2009). Of 36 studies that tested whether a physiological mechanism could explain a correlation between an abiotic variable and a distribution limit,
only 21 (58%) found evidence to support the mechanism; the remainder found partial or no support (Sexton et al., 2009). Publication bias may mean there are additional unpublished “null” results that don’t confirm the prevailing view that species distributions are limited by temperature. The evidence presented here supports an alternative view that range limits are not simple correlations with climate (Helmuth, 2009).

Perhaps this should not come as a surprise. The theorist Robert Holt says that “the range of potential mechanisms limiting species distributions in space is nearly as varied and complex as the diversity of life itself” (Holt et al., 2005). This is certainly true for native species, but it would seem to be less relevant for invasive species, because they are often less encumbered by biotic constraints, and they can benefit from facilitated dispersal (Mack, 2003). This was one of the main rationales for using kudzu in this study to look at the role of temperature in defining plant distributions. Despite their simplified biology, studies that use invasive plants, such as this one, still fail to find a clear temperature limitation, although subtler effects such as interactions with precipitation, biotic limitations or accumulating physiological effects cannot be ruled out. Even if temperature is limiting most species distributions, the point remains that the current view of temperature limitations on species distributions is probably too simplistic. There is potential for greater integration. Holt views the edges of species distributions as “unifying forces” in ecology and evolutionary biology because they integrate most subdisciplines in these fields (Holt and Keitt, 2005).

Holt’s view hints at a way forward. The new subdisciplines of macrophysiology, which looks at large-scale ecological implications of physiology, and organismal climatology, which views climate space through the filter of organismal physiology, are already addressing some of this complexity (Chown et al., 2004; Helmuth et al., 2010). Modelers are starting to recognize, and incorporate biological complexity into their models, but more needs to be done if we are to understand how climate change might influence species distributions. One example of a promising approach is to use “neighbourly advice,” a
Bayesian technique that corrects for micro-climatic variation based on environmental information from several co-occurring species (McInerny and Purves, 2011). This type of analysis could be taken further by constructing a hierarchical Bayesian model that assimilates relevant data from several subdisciplines, such as ecophysiology, population ecology and community ecology (Luo et al., 2011; Jackson et al., 2009). A simpler method that can be implemented immediately is mapping a life history trait, such as growth potential, over distribution limits to test hypotheses about limiting factors. For example, the finding that growth of *Fagus sylvatica* L. in France declines at some range boundaries but not at others should stimulate further experimentation (Seynave et al., 2008). Modeling methods such as these are our best bet for quickly and efficiently increasing our understanding of how species distributions relate to the fundamental niche, but they are only as good as the assumptions and data that inform them (Schenk, 1996). Biologists can help by designing experiments that test key assumptions, and by collaborating with modelers. There have been repeated calls for greater integration of biology into modeling methods (e.g., Wang, 1960; Schenk, 1996; Guisan and Thuiller, 2005); biologists and modelers alike would do well to take note.
Appendix A

Supplemental experiment—the effect of an episodic chill on kudzu growth in summer

A.1 Materials and Methods

In May 2008, the effect of an episodic summer cold spell on stem elongation rate was assessed. Young third-generation clones of three populations (Albany, New York ($N = 3, 42.58^\circ N, 73.77^\circ W$), Mt. Gretna, Pennsylvania ($N = 5, 40.27^\circ N, 76.44^\circ W$), and Huntington Station, New York ($N = 4, 40.81^\circ N, 73.41^\circ W$) were grown in a glasshouse and transferred to one of two identical growth cabinets (Enconair Ecological Chambers, Inc., Model GC–20, Winnipeg, Canada) set to $28^\circ /22^\circ C$, $14/10$ h day/night, and $350–550 \mu$mol$^{-1}$ m$^{-2}$ s$^{-1}$ in May 2008. After a 14 d acclimation period, the temperatures in the cabinets were changed to $15^\circ /5^\circ C$ and $10^\circ /5^\circ C$ for two days, whereupon the temperatures were returned to $28^\circ /22^\circ C$ for five days. The elongation rate of every stem that emerged was measured once 3–4 nodes had formed. Growth rates were not linear in stems less than 6 nodes long (Fig C.1), so they were excluded from subsequent analyses.
Figure A.1 – The effect of an atypical summer chill on growth. Stem elongation rates were measured on potted kudzu plants from three populations treated to a controlled chilling experiment. (A) Maximum and minimum chamber air temperature, showing 2 day chilling treatment to 10°/5°C (blue) and 15°/5°C (black). Lines are offset slightly for visibility. (B) Mean ± se (open circles) and maximum (solid diamonds) growth rates in each treatment (populations pooled). Pre-treatment growth rates (Jul ≤ 142) are summarized in Fig. 3.5.
A.2 Results

Stem elongation rates in potted plants from three northern kudzu populations were subjected to a controlled 2 day chill. The results from the three populations were pooled because they were not significantly different (ANOVA: $F_{2,735} = 1.16$, $P(< |F|) = 0.32$). Prior to the chilling treatment (Jul < 144, Fig. A.1), growth rates were identical ($7.6 \pm 0.2$ cm d$^{-1}$) in both treatments (ANOVA: $F_{1,298} = 0.40$, $P(< |F|) = 0.53$). Starting from the first day of the chill to the end of the experiment (Jul = 144–150, Fig. A.1), growth rates averaged $0.7$ cm d$^{-1}$ slower in the $10^\circ/5^\circ$C treatment than in the $15^\circ/5^\circ$C treatment (ANOVA: $F_{1,436} = 3.94$, $P(< |F|) = 0.048$). On the second day of the chilling treatment, growth rates had slowed to $0.4 \pm 0.1$ cm d$^{-1}$ and $1.0 \pm 0.2$ cm d$^{-1}$ in the $10^\circ/5^\circ$C and $15^\circ/5^\circ$C treatments, respectively (Fig. A.1). Growth rates fully recovered after three days at $28^\circ/20^\circ$C (Fig. A.1). Recovery took longer than observed in the field (Chapter 3, 4), but this is probably due to root chilling, which would not occur in field conditions because the thermal resistivity of soil prevents large fluctuations in temperature (Henry, 2007).
Appendix B

Supplementary material for
Chapter 2

B.1 Supplementary text

B.1.1 Cooling rate and nucleation

Two key considerations in artificial freezing tests are cooling rate and whether to control ice nucleation in the tissue (Gusta, 2003). The influence of cooling rate and nucleation method on measured relative conductivity was examined at the target temperatures of $-12^\circ$, $-16^\circ$ and $-20^\circ$C in a $2 \times 2$ factorial study. The cooling rate of $8^\circ$C h$^{-1}$ was compared with $1^\circ$C h$^{-1}$, and the nucleation method of rinsing was compared to adding ice crystals (Steffen et al., 1989; Gusta, 2003). Plant material was raised in 20 L pots in a roof-top garden in Toronto, ON. Plants were allowed to senesce in the fall and pots remained outside until the experiment. For each of the 12 treatment combinations, 15 stems were harvested from five independent plants, whereby each stem was cut in half for the nucleation treatments. Hypothesis testing using linear models showed that the main effects of nucleation treatment and cooling rate were not significant, but that cooling rate interacted with treatment temperature (data not shown). Cooling by $8^\circ$C h$^{-1}$ to $-12^\circ$C...
resulted in a 0.069 ± 0.014°C increase in relative conductivity ($t_{v=59,\alpha/2=0.025} = 4.864$, $P < 0.001$) relative to 1°C h$^{-1}$, but the effect of cooling rate was not significant at −16 and −20°C. Adding this correction factor to the data resulted in a threshold RC estimate of 0.366, well within the 95% CI [0.292, 0.419] reported here. Because the threshold is not substantially affected, the original estimates were reported.

### B.2 Supplementary figures and tables

**Figure B.1** – Relative conductivity response to treatment temperature for fourteen kudzu populations arranged south to north beginning in the lower left-hand corner of the figure. The population codes in the upper right hand corner of each panel correspond to those in Table 2.1. Each point represents an individual stem sampled randomly from each population.
Table B.1 – ANOVA table of the binomial generalized linear model (clog-log link) fit to stem survival data for pooled above- and belowground stems collected in March 2007 (Fig. 2.3A, Table 2.2), where RC is relative conductivity. Shown are the degrees of freedom (df), deviance, residual df, residual deviance, and the $P$-value. Model AIC = 184.77.

| Term | df | Deviance | Resid. df | Resid. Dev | $P(<|\chi^2|)$ |
|------|----|----------|-----------|------------|----------------|
| NULL | 246| 316.70   | 246       | 316.70     |                |
| RC   | 1  | 135.93   | 245       | 180.77     | $2.06 \times 10^{-31}$ |

Table B.2 – ANOVA table of the binomial generalized linear model (logit link) fit to aboveground stems collected in March 2007 (Table 2.1, Fig. 2.3B). “Temp” is treatment temperature, “Region” is northern or southern populations (Table 2.1), and “:” indicates an interaction between variables. Shown are the degrees of freedom (df), deviance, residual df, residual deviance, and the $P$-value. Model AIC = 146.2.

| Term       | df | Deviance | Resid. df | Resid. Dev | $P(<|\chi^2|)$ |
|------------|----|----------|-----------|------------|----------------|
| NULL       | 231| 72.57    | 231       | 72.57      |                |
| Temp       | 1  | 52.97    | 230       | 19.60      | $3.39 \times 10^{-13}$ |
| Region     | 1  | 3.88     | 229       | 15.72      | 0.049          |
| Temp$^2$   | 1  | 8.61     | 228       | 7.11       | 0.003          |
| Temp:Region| 1  | 1.04     | 227       | 6.07       | 0.309          |
Figure B.2 – Relative conductivity (RC) response to temperature of aboveground stems collected on (A) Nov 12, 2009, (B) Feb 7, 2010, (C) Mar 21, 2010, and on (D) May 30, 2010 in Leamington, Ontario, Canada (42.02°N, 82.74°W). Points are mean RC ± se of 3–9 stem samples per temperature at the four time points. The solid line is the generalized linear model $RC = Temp + Type + Month + Temp:\text{Type} + Temp:\text{Month} + Month:\text{Temp}^2 + Type:\text{Month} + Type:\text{Temp}^2$ (Table B.4) with the 95% CI (dashed lines), where “:” indicates an interaction between variables. The critical temperature shown in Fig. 2.6 is where the lethal 36% RC from Fig. 2.3A (dotted line) intersects the model and the CI.
Figure B.3 – Relative conductivity (RC) response to temperature in roots collected in Leamington, Ontario, Canada on (A) Nov 12, 2009, (B) Feb 7, 2010, (C) Mar 21, 2010, and in (D) May 30, 2010. Points are mean RC ± se of 3–7 samples (except in Feb: 1–3) per temperature. The solid line is the generalized linear model RC = Temp + Type + Month + Temp:Type + Temp:Month + Type:Month + Month:Temp^2 + Type:Temp^2 (Table B.4) with the 95% CI (dashed lines), where “:” indicates an interaction between variables. The critical temperature shown in Fig. 2.6 is where the lethal 36% RC from Fig. 2.3A (dotted line) intersects the model and the CI.
Table B.3 – ANOVA table of the binomial generalized linear model (logit link) fit to aboveground and belowground stems (crowns) collected in Beltsville, Maryland in March 2007 (Table 2.1). The belowground portion of this model is shown fit to the data in Fig. 2.3C. “Temp” is treatment temperature, “Type” is aboveground or belowground, and “:” indicates an interaction between variables. Shown are the degrees of freedom (df), deviance, residual df, residual deviance, and the $P$-value. Model AIC = 23.7.

<table>
<thead>
<tr>
<th>Term</th>
<th>df</th>
<th>Deviance</th>
<th>Resid. df</th>
<th>Resid. Dev</th>
<th>$P(&lt;\chi^2)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NULL</td>
<td></td>
<td>27</td>
<td>11.46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temp</td>
<td>1</td>
<td>7.77</td>
<td>26</td>
<td>3.70</td>
<td>0.005</td>
</tr>
<tr>
<td>Type</td>
<td>1</td>
<td>1.05</td>
<td>25</td>
<td>2.64</td>
<td>0.305</td>
</tr>
<tr>
<td>Temp$^2$</td>
<td>1</td>
<td>0.83</td>
<td>24</td>
<td>1.81</td>
<td>0.362</td>
</tr>
<tr>
<td>Temp:Type</td>
<td>1</td>
<td>0.27</td>
<td>23</td>
<td>1.53</td>
<td>0.602</td>
</tr>
</tbody>
</table>
Table B.4 — ANOVA table of the binomial generalized linear model (logit link) fit simultaneously to aboveground stems, belowground stems, and roots from Leamington, Ontario, Canada (Figs. 2.5, B.2, B.3). “Temp” is treatment temperature, “Type” is the tissue type, “Month” is collection time, and “:” indicates an interaction between variables. Shown are the degrees of freedom (df), deviance, residual df, residual deviance, and the $P$-value. Model AIC = 523.74.

| Term       | df | Deviance | Resid. df | Resid. Dev | $P(<|\chi^2|)$ |
|------------|----|----------|-----------|------------|----------------|
| NULL       | 747| 339.51   |           |            |                |
| Temp       | 1  | 98.53    | 746       | 240.98     | $1.02 \times 10^{-22}$ |
| Type       | 2  | 44.13    | 744       | 196.86     | $2.52 \times 10^{-10}$ |
| Month      | 3  | 88.28    | 741       | 108.58     | $2.05 \times 10^{-19}$ |
| Temp:Type  | 2  | 20.90    | 739       | 87.68      | $2.68 \times 10^{-5}$ |
| Temp:Month | 3  | 2.24     | 736       | 85.44      | 70.52          |
| Type:Month | 6  | 8.69     | 730       | 76.76      | 0.19           |
| Month:Temp$^2$ | 4 | 7.85   | 726       | 68.91      | 0.10           |
| Type:Temp$^2$ | 2 | 1.63   | 724       | 67.28      | 0.44           |
Table B.5 – Maximum likelihood estimates of critical temperatures (°C) for the four collections in Leamington, Ontario, Canada (sampled in 2009–2010, Fig. 2.6), and for the seven northern and seven southern populations (sampled in March 2007, Fig. 2.4). 2007 belowground stems were sampled only in Beltsville, Maryland, USA (Table 2.1, Fig. 2.3C). Estimates were obtained by solving the link functions of models fit to the Leamington data (Table B.4), the 2007 aboveground stem data (Table B.2), and the 2007 belowground stem data (Table B.3) for the critical temperature that results in the lethal level of 36% RC (Fig. 2.3A). Interaction between variables is indicated by “:”. The 95% CI is in brackets.

<table>
<thead>
<tr>
<th>Location</th>
<th>Collection</th>
<th>Belowground stems</th>
<th>Aboveground stems</th>
<th>Roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leamington</td>
<td>Mar 21, 2010</td>
<td>−10.8 [−3.9, −16.4]</td>
<td>−23.2 [−18.8, −28.0]</td>
<td>−5.6 [−2.4, −8.7]</td>
</tr>
<tr>
<td>Leamington</td>
<td>May 30, 2010</td>
<td>−2.5 [1.8, −6.8]</td>
<td>1.1 [&gt; 4, −3.5]</td>
<td>−3.4 [−0.5, −6.6]</td>
</tr>
<tr>
<td>Southern states</td>
<td>Mar 2007</td>
<td>NA</td>
<td>−14.4 [−10.6, −17.7]</td>
<td>NA</td>
</tr>
</tbody>
</table>
Table B.6 – Comparison of air temperature and stem temperature of shaded, hanging kudzu stems in March 2007 at five U.S. kudzu populations. Instantaneous temperatures were measured using a 36-gauge thermocouple (Type T, Omega, Laval, QC) attached to a LI-1000 datalogger (LI-COR, Lincoln, NE). Air temperatures are mean ± sd of 8–10 readings of a 36-gauge thermocouple inserted into a radiation shield equilibrated to air temperature and suspended at approximately 2 m within the kudzu stand. Stem temperatures are the mean ± sd of 8–10 stems within a few meters of the air temperature location.

<table>
<thead>
<tr>
<th>Location</th>
<th>Air, °C ± sd</th>
<th>Stem, °C ± sd</th>
<th>Air–Stem, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valdosta, GA</td>
<td>22.87 ± 0.19</td>
<td>23.92 ± 0.49</td>
<td>−1.05</td>
</tr>
<tr>
<td>Statesboro, GA</td>
<td>22.25 ± 0.04</td>
<td>21.90 ± 0.30</td>
<td>0.35</td>
</tr>
<tr>
<td>Beltsville, MD</td>
<td>18.65 ± 0.06</td>
<td>17.99 ± 0.54</td>
<td>0.66</td>
</tr>
<tr>
<td>Holly Springs, MS</td>
<td>13.04 ± 0.19</td>
<td>12.75 ± 0.62</td>
<td>0.29</td>
</tr>
<tr>
<td>Vicksburg, MS</td>
<td>15.75 ± 0.14</td>
<td>15.36 ± 0.41</td>
<td>0.39</td>
</tr>
<tr>
<td>Narrows of the Harpeth, TN</td>
<td>7.72 ± 0.25</td>
<td>8.92 ± 0.51</td>
<td>−1.19</td>
</tr>
</tbody>
</table>
Appendix C

Supplementary material for
Chapter 3
Figure C.1 – Stem elongation rate of stems as a function of the number of nodes back from the tip. The fourth node is usually the first expanding leaf. Measurements were made from the marked node to the tip with a flexible seamstress tape during the pre-treatment phase of the May 2008 controlled chilling experiment (Fig. A.1).
Table C.1 – Leaf temperature in the gas exchange cuvette and maximum daily air temperature during spring 2010 and 2011. Air temperatures were recorded at Harrow meteorological station (details in Table 3.1, www.climate.weatheroffice.gc.ca).

<table>
<thead>
<tr>
<th>Year</th>
<th>Date</th>
<th>No. of leaves</th>
<th>Leaf temperature in cuvette, °C, mean ± se</th>
<th>Maximum air temperature at Harrow, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>May 5</td>
<td>12</td>
<td>30.8 ± 0.8</td>
<td>26.4</td>
</tr>
<tr>
<td></td>
<td>May 6</td>
<td>12</td>
<td>25.2 ± 0.1</td>
<td>18.7</td>
</tr>
<tr>
<td></td>
<td>May 9</td>
<td>12</td>
<td>18.7 ± 0.2</td>
<td>12.9</td>
</tr>
<tr>
<td></td>
<td>May 10</td>
<td>11</td>
<td>24.0 ± 0.7</td>
<td>11.9</td>
</tr>
<tr>
<td></td>
<td>May 20</td>
<td>5</td>
<td>28.2 ± 0.7</td>
<td>25.1</td>
</tr>
<tr>
<td></td>
<td>May 20 (new leaves)</td>
<td>11</td>
<td>28.0 ± 0.3</td>
<td>25.1</td>
</tr>
<tr>
<td></td>
<td>May 30 (new leaves)</td>
<td>10</td>
<td>31.5 ± 0.2</td>
<td>28.2</td>
</tr>
<tr>
<td>2011</td>
<td>May 14</td>
<td>12</td>
<td>24.9 ± 0.04</td>
<td>18.7</td>
</tr>
<tr>
<td></td>
<td>May 17</td>
<td>8</td>
<td>21.0 ± 0.2</td>
<td>11.2</td>
</tr>
<tr>
<td></td>
<td>May 18</td>
<td>10</td>
<td>22.5 ± 0.2</td>
<td>17.4</td>
</tr>
<tr>
<td></td>
<td>May 21</td>
<td>8</td>
<td>27.3 ± 0.3</td>
<td>23.9</td>
</tr>
<tr>
<td></td>
<td>May 28</td>
<td>7</td>
<td>23.0 ± 0.2</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>May 28 (new leaves)</td>
<td>6</td>
<td>25.1 ± 0.3</td>
<td>20.0</td>
</tr>
</tbody>
</table>
Figure C.2 – Hourly air temperature in 2010 and 2011 measured at the Harrow meteorological station (www.climate.weatheroffice.gc.ca). Arrows show the first day of measurements each year.
Table C.2 – Difference between leaf temperature logged at 20 min intervals on three leaves in the middle of the slope, and air temperature at the Harrow meteorological station. Leaf temperatures were measured with 36–gauge thermocouples (Type T, Omega, Laval, QC) connected to a datalogger (VL–1700, Veriteq Instruments, Richmond, BC). Thermocouples were attached to the underside of leaves with porous paper medical tape. Daily mean temperatures are shown, along with the minimum and maximum daily temperature.

<table>
<thead>
<tr>
<th>Date</th>
<th>Air temperature, °C</th>
<th>Leaf temperature, °C</th>
<th>Leaf–air difference in minimum temperature, °C</th>
<th>Leaf–air difference in maximum temperature, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 10, 2011</td>
<td>15.2 [10.9, 19.4]</td>
<td>16.5 [11.7, 29.5]</td>
<td>0.8</td>
<td>10.1</td>
</tr>
<tr>
<td>May 12, 2011</td>
<td>19 [13.4, 24.9]</td>
<td>18.4 [12.6, 30.2]</td>
<td>−0.8</td>
<td>5.3</td>
</tr>
<tr>
<td>May 13, 2011</td>
<td>20.1 [14.6, 25.6]</td>
<td>19.2 [12.0, 32.0]</td>
<td>−2.6</td>
<td>6.4</td>
</tr>
<tr>
<td>May 15, 2011</td>
<td>9.5 [6.3, 12.7]</td>
<td>10.2 [6.5, 12.5]</td>
<td>0.2</td>
<td>−0.2</td>
</tr>
<tr>
<td>May 16, 2011</td>
<td>7.9 [5.6, 10.1]</td>
<td>8.2 [6.2, 14.2]</td>
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<td>4.1</td>
</tr>
<tr>
<td>May 17, 2011</td>
<td>9.6 [8.0, 11.2]</td>
<td>8.0 [7.1, 10.0]</td>
<td>−0.9</td>
<td>−1.2</td>
</tr>
</tbody>
</table>
Figure C.3 – Mean stem elongation rate as a function of stem diameter above the third node from the base of the stem. Each point is the growth rate of a single stem averaged over the entire study 2011 study period in Leamington, Ontario. Only stems that were measured on every day of the study are included. The line is the linear model $y = 2.3x - 3.3$, adj. $R^2 = 0.90$. 
Table C.3 – Comparison of linear models fit to growth rate measurements. Adjusted R² is the proportion of variance explained, adjusted for the number of parameters (larger values = better model), while the Akaike’s Information Criterion (AIC) is a likelihood-based (L) measure of fit (AIC = 2k − 2ln(L), smaller values = better model). On days where measurements were also taken the day before, “period” averages, absolutes, and “day before” values are all identical.

<table>
<thead>
<tr>
<th>Temperature variable</th>
<th>adj. R²</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period mean maximum</td>
<td>0.43</td>
<td>1790</td>
</tr>
<tr>
<td>Period mean</td>
<td>0.41</td>
<td>1801</td>
</tr>
<tr>
<td>Period absolute maximum</td>
<td>0.38</td>
<td>1823</td>
</tr>
<tr>
<td>Period mean minimum</td>
<td>0.34</td>
<td>1849</td>
</tr>
<tr>
<td>Day before mean</td>
<td>0.31</td>
<td>1869</td>
</tr>
<tr>
<td>Day before minimum</td>
<td>0.31</td>
<td>1874</td>
</tr>
<tr>
<td>Day before maximum</td>
<td>0.24</td>
<td>1908</td>
</tr>
<tr>
<td>Day of mean</td>
<td>0.23</td>
<td>1911</td>
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<tr>
<td>Period absolute minimum</td>
<td>0.20</td>
<td>1927</td>
</tr>
<tr>
<td>Day of minimum</td>
<td>0.20</td>
<td>1931</td>
</tr>
<tr>
<td>Day of maximum</td>
<td>0.20</td>
<td>1931</td>
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<tr>
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<td>2021</td>
</tr>
</tbody>
</table>
**Table C.4** – ANOVA table of a linear model describing net CO₂ assimilation rate ($A_{\text{net}}$) as a function of measurement ($T_{\text{meas}}$) temperature, and minimum morning temperature ($T_{\text{min}}$). $T_{\text{min}}$ is parameterized as a discrete variable with three levels: $T_{\text{min}} < 10^\circ \text{C}$, $T_{\text{min}} = 10$–$12.5^\circ \text{C}$, and $T_{\text{min}} > 12.5^\circ \text{C}$. Data is shown in Fig. 3.6. Rates were measured in Leamington in spring 2010 and 2011. The model is $A_{\text{net}} = \beta_0 + \beta_1 T_{\text{meas}} + \beta_2 T_{\text{min}} + \beta_3 T_{\text{meas}}:T_{\text{min}}$ (adj. $R^2 = 0.80$), where “:” indicates an interaction between variables.

| Term      | df | SS    | MS    | $F$   | $P(< |F|)$ |
|-----------|----|-------|-------|-------|-----------|
| $T_{\text{meas}}$ | 1  | 1007.9| 1007.9| 154.1 | < 0.001   |
| $T_{\text{min}}$  | 2  | 1509.6| 754.8 | 115.4 | < 0.001   |
| $T_{\text{meas}}:T_{\text{min}}$ | 2  | 17.7  | 8.9   | 1.4   | 0.26      |
| Residuals  | 88 | 575.7 | 6.5   |       |           |
Appendix D

Supplementary material for

Chapter 4
Figure D.1 – Daily air temperature at Harrow meteorological station (www.climate.weatheroffice.gc.ca) (A, C) and stem elongation rates measured in the field in Leamington, Ontario (B, D). Mean (solid line), minimum and maximum (dashed lines) daily air temperature in June (A) and July (C) 2011. (B) Mean ± se (open circles) and maximum (closed diamonds) stem elongation rates of 20 marked stems. Stem elongation rates are also shown in Fig. 4.1. Kate Henbest collected these data under the supervision of H. Coiner.

Figure D.2 – Stem elongation rates measured on potted kudzu plants from two populations grown in cool (22/14°C, blue symbols) and warm (31/22°C, black symbols) conditions. Mean ± se (open circles) and maximum (closed diamonds) growth rates in each treatment (populations pooled). Data is summarized in Fig. 4.1.
Table D.1 – Comparison of linear models fit to growth rate measurements. Adjusted $R^2$ is the proportion of variance explained, adjusted for the number of parameters (larger values = better model), while the Akaike's Information Criterion (AIC) is a likelihood-based measure of fit ($AIC = 2k - 2ln(L)$, smaller values = better model). On days where measurements were also taken the day before, “period” averages, absolutes, and “day before” values are all identical. On two of the seven days represented here, there were two days between measurements, so all variables were retained.

<table>
<thead>
<tr>
<th>Temperature variable</th>
<th>adj. $R^2$</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period mean maximum</td>
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<td>1146</td>
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<tr>
<td>Period absolute maximum</td>
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<tr>
<td>Day before maximum</td>
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<td>1151</td>
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<tr>
<td>Period mean</td>
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<td>Day before mean</td>
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<td>Day of minimum</td>
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</tr>
<tr>
<td>Day before minimum</td>
<td>0.34</td>
<td>1168</td>
</tr>
<tr>
<td>Period mean minimum</td>
<td>0.33</td>
<td>1169</td>
</tr>
<tr>
<td>Day of mean</td>
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<td>1174</td>
</tr>
<tr>
<td>Period absolute minimum</td>
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<td>1179</td>
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<tr>
<td>Day of maximum</td>
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<tr>
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</table>
Appendix D. Supplementary material for Chapter 4

Figure D.3 – Representative diurnal response of photosynthesis parameters measured in situ on June 13, 2011. (A) Air (solid line) and leaf (open symbols) temperature, (B) ambient (closed symbols) and incident (open symbols) irradiance, (C) net CO$_2$ assimilation rate, (D) stomatal conductance, (E) the ratio of intercellular to atmospheric CO$_2$ ($C_i/C_a$), and (F) air-leaf vapor pressure difference (VPD) of a representative leaf, measured repeatedly at two-hour intervals. Cuvette conditions were changed to match ambient conditions.
Figure D.4 – Representative diurnal response of photosynthesis parameters measured in situ on June 14, 2011. (A) Air (solid line) and leaf (open symbols) temperature, (B), ambient (closed symbols) and incident (open symbols) irradiance, (C) net CO₂ assimilation rate, (D) stomatal conductance, (E) the ratio of intercellular to atmospheric CO₂ ($C_i/C_a$), and (F) air–leaf vapor pressure difference (VPD) of a representative leaf, measured repeatedly at two-hour intervals. Cuvette conditions were changed to match ambient conditions.
Figure D.5 – Relationship between leaf expansion rate and stem elongation rate, measured in the field in Leamington, Ontario in June 2011. The regression line is $y = 0.06x + 0.4$, adj. $R^2 = 0.57$, $(F_{1,28} = 39.5, P(<|F|) \leq 0.001)$. Stem elongation rates are also shown in Figs. D.1B and 4.1. Kate Henbest collected these data under the supervision of H. Coiner.
Table D.2 – ANOVA table of a linear model describing average stem elongation rate as a function of stem diameter and time of year. Rates were measured in Leamington in June and July 2011. The model is Rate = $\beta_0 + \beta_1$Diameter + $\beta_2$Month, where $\beta_0 = -3.0$, $\beta_1 = 2.7$, and $\beta_2 = 4.7$, adj. $R^2 = 0.73$.

| Term            | df | SS   | MS   | $F$   | $P(< |F|)$ |
|-----------------|----|------|------|-------|-----------|
| Diameter        | 1  | 650.7| 650.7| 108.26| < 0.001   |
| Month           | 1  | 307.9| 307.9| 51.22 | < 0.001   |
| Diameter:Month  | 1  | 7.10 | 7.10 | 1.18  | 0.282     |
| Residuals       | 56 | 336.7| 6.0  |       |           |
Appendix E

Supplementary material for
Chapter 5
**Figure E.1** – Stem elongation rate as a function of mean maximum temperature over the 1–11 day period pertaining to the measurement. Open symbols are the overall means, solid symbols are means of the fastest quintile (20%) and crossed symbols are the means of the slowest quintile. Growth rates on each day of measurement on the roof of the Earth Sciences Centre in summer 2010 (circles), fall 2008 (diamonds), and fall 2009 (squares) (Figs. 5.6, 5.7, 5.8). Regression lines are fit to all three datasets simultaneously. On two days in 2008, there was insufficient data to calculate the quintiles, so just the means are shown. The regression for the upper and lower quintiles are \( y = 0.86x - 13.0 \) (adj. \( R^2 = 0.68 \)) and \( y = 0.28x - 5.3 \) (adj. \( R^2 = 0.55 \)), respectively. The line fit to summer and fall mean growth rates is \( y = 0.52x - 8.6 \) (adj. \( R^2 = 0.52 \)). The \( x \)-intercepts of the extrapolated lines predict zero growth will occur between 15°C and 19°C. To facilitate comparison with measurements later in the season, the scale is the same as Figs. 4.1 and 3.5.
References


REFERENCES


Bargeron, C., 2009. Early detection and distribution mapping system. Center for Invasive Species and Ecosystem Health, University of Georgia.


REFERENCES


References


Godsoe, W., 2010. I can’t define the niche but I know it when I see it: a formal link between statistical theory and the ecological niche. *Oikos* 119:53–60.


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