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<th>Journal:</th>
<th>Canadian Journal of Forest Research</th>
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<tr>
<td>Manuscript ID:</td>
<td>cjfr-2016-0537.R1</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>Review</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>07-Mar-2017</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Despland, Emma; Université Concordia</td>
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<tr>
<td>Keyword:</td>
<td>Lepidoptera, hatchlings, synchrony, outbreaks, larvae</td>
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EFFECTS OF PHENOLOGICAL SYNCHRONIZATION ON CATERPILLAR
EARLY-INSTAR SURVIVAL UNDER A CHANGING CLIMATE

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Abstract
Early instar caterpillars experience very high and often very variable mortality; if it is density-dependent, it can be a key factor in outbreak dynamics.

Plant physical and chemical defenses can be extremely effective against young caterpillars, even of specialists. Phenological asynchrony with host plants can lead to dispersal and mortality in the early instars, increased predation or poor nutrition in later instars. Predation on early-instar larvae (including cannibalism) can be extremely high, parasitism appears generally low, and pathogens acquired early in larval development can lead to high mortality in later stadia.

Four well-studied species reveal very different roles of early-instar mortality in population dynamics. In spruce budworm and gypsy moth, early-instar mortality rates can be very high; they do not drive outbreak cycles, because density-dependence is weak, but can modulate cycles and contribute to outbreak size and duration. For the autumnal moth, early-instar survival depends on host plant synchrony, but may or may not be density-dependent. For monarch butterflies, the relative importance of larval mortality rates in population dynamics remains unclear.

Tritrophic interactions between herbivores, host plants, natural enemies and microbes play complex and species-specific roles in early-instar ecology, leading to emergent dynamics in population fluctuations. The phenology of these relationships is often poorly understood, making their responses to climate change unpredictable.

Key words: Lepidoptera, hatchlings, synchronization, outbreaks
“...plague and predation are part of the warp and woof of nature....The tent caterpillars are no exception. ... The colony that had welcomed spring 200 hundred strong had been reduced to half its number. The enormous population of tent caterpillars had been cropped by weather, starvation, ants, bugs, parasites, fungi, viruses, bacteria, and misadventure in general. It had been a particularly trying year. Summer had hardly begun and the die had already been cast for the year to come. There would be fewer moths, fewer egg masses, and fewer colonies in the next generation. As with all species, not excluding mankind, the reproductive potential, the birth rate, genetically built in, generated an inexorable tide. Only the competing tides of multitudinous species all striving for their share of the finite materials of the planet keep the flood of life at an even level. The flood was never placid, and the waves, sometimes ripples, sometimes huge swells, reflected the fierce underlying competitive struggles. The rise and fall of the tent caterpillar populations from one year to the next, from one phase of a cycle to another, was just one facet mirroring the perpetual competition.” (Dethier 1980)

INTRODUCTION
Most Lepidoptera are r-selected, meaning that populations are governed by maximum reproductive capacity. Large numbers of eggs are laid in each generation, but survival rate is low. The high number of fragile offspring means that population growth rate can vary tremendously with survival rate of those offspring, generating dramatic fluctuations in population size. Phenology mediates the interactions between herbivores, host plants and natural enemies and can therefore play a key role in determining survival rates. A changing climate can
perturb these phenological relationships, with potentially dramatic consequences for population sizes (Parmesan 2006; van Asch and Visser 2007; Boggs 2016). The current paper will explore the vulnerability of neonate larvae to these phenological mismatches and its role in population dynamics under climate change.

**E A R L Y− I N S T A R B I O L O G Y**

Neonate caterpillars face several considerable challenges upon hatching: they need to overcome plant mechanical (e.g. spines, trichomes, surface waxes, tough cuticles) and chemical defenses (including secretions) and initiate feeding, before exhausting the resources with which their mothers provisioned their eggs, and without being dislodged from the plant by wind and rain or falling prey to natural enemies.

Early instar caterpillars are not merely smaller versions of their final-instar selves, they not only differ in scaling properties (e.g. mandible size and strength, thermal mass, surface to volume ratio) but also in metabolism, digestive physiology, sensory modalities (Hochuli et al. 2001) and gut microbiota (Mason and Raffa 2014). In general, young caterpillars have higher relative growth rates, consumption rates, metabolic rates, and assimilation efficiency, but lower net growth efficiency, than older ones (Scriber and Slansky Jr 1981, Johnson and Zalucki 2007). They are more selective of food sources and more sensitive to plant chemistry, possibly due to a more limited array of digestive enzymes (Hochuli et al. 2001). They are hence often more vulnerable to plant compounds and pathogens that have little effect on older individuals (Zalucki et al. 2002). These small larvae usually have limited vision, but are often positively phototactic and negatively
geotactic, and also respond to olfactory and gustatory cues (Perkins et al. 2008).

Many species start out cryptic, acquiring warning colouration later in development, and many others are gregarious in the early instars, becoming solitary as selection pressures change with increasing size (Despland 2013).

**Mortality Rates of Early Instars**

In the normal course of events, r-selection implies that most individuals die. Indeed, studies on Lepidopteran population dynamics show that the early instars experience huge attrition (Zalucki et al. 2002), such that type III survivorship curves (with most mortality occurring in the early stages) are the most common in Lepidopteran life histories (Floater and Zalucki 1999).

For instance, among forest defoliators, one study shows forest tent caterpillars, *Malacosoma disstria*, to experience 61-66% mortality in the first three larval instars in aspen and mixedwood stands in Alberta, Canada (Nixon and Roland 2012). At the other end of the world, on acacias in Queensland, Australia, the gregarious processsionary caterpillar *Ochrogaster lunifer* experienced over 50% mortality in the first larval stage across all populations studied, with 82% of colonies wiped out before the end of larval development (Floater and Zalucki 1999). In both cases, the most important cause of early instar mortality was arthropod predation.

Among agricultural pests, a *Pieris rapae* life table shows 61% mortality during the first instar mostly due to predation and drowning, with parasitoids gaining in importance later in larval development (Dempster 1967). Codling moth, *Cydia pomonella*, first-instar larvae on pear suffered 55% mortality in the first larval
instar, linked to lignification preventing larval entry to the fruit (Westigard et al. 1976).

Non-pest butterflies have received less attention, but appear to suffer similar high attrition rates: in the first two days post-hatch, *Heliconius* caterpillars feeding on neotropical *Passiflora* vines experienced 70% mortality on plants without ants attending extrafloral nectaries, but 45% on ant-attended plants (Smiley 1985). The sulphur butterfly *Colias alexandra* in montane valleys in the Rockies suffered between 37-52% mortality in the first instar and 32-60% in the second, averaging 90% in the first three pre-diapause instars, attributed mainly to failure to establish feeding or dislodgment from the host plant (Hayes 1981).

A review by Zalucki et al (2002) shows that mortality in the early instars is often not only very high but also extremely variable. Notably it does not identify any clear patterns with life history traits: there is no obvious difference between concealed and open feeders, batch or solitary laid eggs, specialist vs polyphagous feeders. Variance is often so high, even within species, between years, sites or host plants that it is difficult to generalize about the adaptive value of different traits (Zalucki et al. 2002). This high variability also implies volatile population dynamics, where endemic populations, undetectable in the early instars, can cause heavy defoliation only weeks later if unusually large numbers survive to the later instars.

Population processes clearly depend on survival at all life stages. Successful management or conservation requires identifying key stage(s) influencing
population dynamics. Life history stages with density-dependent mortality will be important in regulating population size: if density dependence is direct (mortality increases with increasing population size) it will tend to stabilize populations, but lagged (i.e. higher order) density dependent mortality tends to increase population fluctuations and even generate outbreaks (Cooke et al. 2007). Understanding rates and causes of mortality at all life history stages thus has practical implications for managing population dynamics.

**Sources of Early Instar-Mortality**

The small size of first instar caterpillars implies that they can be dislodged from the host plant by a falling raindrop (personal observation). Most life table studies include a substantial category labelled “unkown” or “disappeared” which likely reflects the high frequency of such accidents.

‘Failure to establish on plant’ is often cited as a cause of early-instar mortality; the explanatory value of this observation is limited, but it is likely linked to plant defensive traits. Spines and trichomes can prevent small larvae from accessing the leaf surface (Rathcke and Poole 1975), toughness can prevent small mandibles from piercing the cuticle (Clissold et al. 2009) and secretions can mire and drown neonates (Zalucki et al. 2001b). For instance, leaf area consumption of Siberian elm clones by neonate *Paleacrita vernata* was negatively related to the trichome density (Dix et al. 1996). Plant chemical defenses can decrease food consumption, decrease growth rate, cause catalepsis or simply death (Scriber and Slansky Jr 1981).
Predation and parasitism have often been thought to be more important in later larval instars (Hawkins et al. 1997), but it is increasingly recognized that mortality of young larvae is often underestimated because they are so small and difficult to study. Indeed, an increasing body of research suggests that predation rates on early-instar larvae can be very high (Zalucki et al. 2002, Nixon and Roland 2012). Cannibalism, of eggs by neonates and of neonates by older larvae, can be an important source both of mortality and of nutrition, and is influenced by environmental factors including population density, food nutritional quality (in particular protein content) and synchrony with the host plant (Richardson et al. 2010). Parasitism appears to be generally low for young instars, but it can be very high on eggs, and since mortality due to parasites is usually delayed, it can be difficult to establish which stage was attacked.

Finally, increasing numbers of studies highlight the effects of microbes (as pathogens or symbionts) on insect performance. For instance, leaf surface microflora can affect herbivores and the scant evidence that exists suggests that pathogens from the phylloplane are more harmful for young than older caterpillars: (Leong et al. 1997). However, the population level consequences of these interactions remain unresolved.

**Phenological Relationships Under a Changing Climate**

The importance of phenological synchrony between a herbivore’s feeding stages and the availability of high quality plant tissue has long been recognized (Feeny 1970), and has received renewed interest in this era of changing climate (Parmesan 2006).
Indeed, the timing between insect feeding stages and the deployment of plant defences can be critical for insect survival and development (van Asch and Visser 2007); notably many insects rely on young expanding foliage, which is generally less tough and more nutritious and hence provides a window of opportunity for insect herbivores (e.g. temperate zone hardwoods (Feeny 1970; Hunter and Elkington 2000); boreal conifers (Lawrence 1997); tropical shade-tolerant angiosperms (Kursar and Coley 2003)). These phenological mismatches mostly affect fragile young larvae.

For instance, 69% of Rhopobota naevana neonates die before feeding when presented with only old cranberry leaves, compared to 11% on young expanding foliage. Population growth is thus 5 times higher if larvae hatch in synchrony with host budburst (Cockfield and Mahr 2013). First instar Zeiraphera canadensis show dramatically lower survivorship (from 50-70% to less than 25%) when fed on 4-5 day old white spruce foliage; in the field, these larvae move in the tree, tracking changes in foliar quality following bud-burst away from the most vigorous apical shoots to those with more immature foliage (Carroll and Quiring 1994).

Synchrony with host plant has been suggested as an important factor in the dynamics of many forest pests (see review by (van Asch and Visser 2007)). The role of host synchrony can be difficult to ascertain as many larvae can disperse away from poorly-synchronized hosts to phenologically suitable ones if they are available within a reasonable distance (e.g. Malacosoma disstria, (Donaldson and Lindroth 2008)). This implies that the window of opportunity defined by host phenology is spatial as well as temporal, and consideration of distribution of host
phenologies across the landscape is essential to understanding their role in
defoliator population dynamics (Foster et al. 2013).

Past debates on the strength of terrestrial trophic cascades, or the relative
importance of top-down and bottom-up effects in regulating defoliator dynamics
(Schmitz et al. 2004) are increasingly recognized as sterile, because host plant traits,
natural enemies, microbes and environmental often interact in ways that are
complex and species specific, generating unpredictable emergent dynamics that can
play a key role in outbreaks (Cooke et al. 2007, Stam et al. 2014). These tritrophic
interactions can lead, for instance, to the slow-growth-high-mortality scenario
whereby slow development on poor quality (or poorly-synchronized) host plants
leads to higher predation in later instars as caterpillars fail to grow large enough to
escape attack before the natural enemies become abundant (e.g. Malacosoma
disstria (Parry et al. 1998)). This complexity implies that the natural history of
individual study systems must be taken into consideration in order to understand
how phenology mediates multi-trophic interactions and their consequences for
early-instar survivorship. These relationships are explored through four different
case studies, chosen for their differences in natural history, phenological
relationships, population dynamics and responses to climate change.

EARLY INSTAR MORTALITY AND POPULATION DYNAMICS –
FOUR CASE STUDIES

SPRUCE BUDWORM
The spruce budworm (Choristoneura fumiferana) is the most important defoliator
of conifers in the Canadian boreal forest. The last major outbreak peaked in the
1970s and damaged or killed trees across nearly 58 million hectares, resulting in the loss of about 500 million cubic metres of timber and costing some $12.5 billion in Québec alone in lost revenue in remote areas with few other industries. On Cape Breton Island (NS), 71% of merchantable timber was destroyed. A new outbreak is currently underway on Québec’s north Shore and spreading in Gaspésie (Sonntag 2016).

The main regulator of budworm outbreaks is thought to be parasitism on late instar larvae. A ca. 40 year intrinsic oscillation in population density is generated by lagged density-dependent changes in survival of late-larvae and pupae, most likely caused by natural enemies (Royama 1984). This oscillation is well-documented, but is imperfectly periodic due to modulation by other secondary factors (Cooke et al. 2007), including variation in early-instar survival (Royama 1984, Régnière and Nealis 2008, Bouchard and Auger 2014).

First instar spruce budworm larvae hatch from the egg in late summer and, without feeding, molt to the second instar and move to a sheltered spot to build a hibernaculum in which to overwinter. In the spring, budworm that emerge before host budbreak mine old needles (Blais 1952, Trier and Mattson 1997). Recent work suggests that these old needles are difficult to pierce but provide a nutritious food source to those caterpillars that succeed (Fuentealba Morales et al. 2017). When budbreak occurs, larvae move to the bud and complete their development on the high-nutrient expanding foliage. High quality foliage during bud elongation thus defines a window of opportunity for the budworm (Lawrence et al. 1997).
Early-instar spruce budworm larvae experience extreme variation in mortality rates: for example, survival rate from the first to the third instar declined over the course of an outbreak from 73% (1984) to 4% (1996) at one study site (Régnière and Nealis 2007). Stand-scale life tables have suggested that early instar survival is linked to variance in the timing of emergence from diapause relative to host budbreak, to the duration of the needle mining period prior to budbreak, to prior defoliation and to infection with microsporidian parasites (Régnière and Nealis 2008).

One key piece of the puzzle is early spring dispersal by ballooning of second-instar larvae emerging from diapause. Ballooning, or drifting in the wind suspended on a fine silk thread, is very risky and associated mortality is thought to be very high (Johns and Eveleigh 2013), but is almost impossible to measure in a forest setting. Dispersal and associated mortality depend on bud availability to emerging larvae, and therefore are thought to increase with phenological mismatch. In this context, variation in the timing of emergence from diapause can be interpreted as a bet-hedging strategy, to ensure that at least some progeny are adequately synchronised with the host plant (Régnière and Nealis 2008). Spring temperature can thus impact early-instar survival via effects on phenological synchrony with the host plant, and landscape-scale analysis of an outbreak showed a significant role for spring temperatures in predicting population growth (Bouchard & Auger 2014). This weather effect is independent of population density; however, it can play an important role in the spatial distribution of outbreaks. Indeed, the Northern distribution of the current outbreak is thought to be linked to improved survival on
black spruce, a more northern host plant, due to phenological advances in budbreak linked to climate warming (Pureswaran et al. 2015).

Bud availability (and hence early-instar survival) also decreases with prior defoliation, as severely damaged trees produce fewer buds the following season (Blais 1952, Régnière and Nealis 2008, Johns and Eveleigh 2013). This bottom-up effect of defoliation on early-instar survival is a lagged, density-dependent process and therefore can have a significant influence on the duration and severity of outbreaks (Cooke et al. 2007).

Finally, although viral and bacterial epizootics are not thought to significantly affect budworm population dynamics, infection with Nosema (a microsporidian parasite) is common in outbreak populations. Nosema is generally sublethal and vertically transmitted through the egg. Maternal infection leads to lower overwinter survival, later emergence, later dispersal and lower success in establishing a feeding site in young larvae (van Frankenhuyzen et al. 2007). In general, natural enemies seem to play only a minor role in early-instar budworm survival, and the factors large-scale studies isolate as the strongest predictors are spring weather and prior defoliation (Régnière and Nealis 2008, Bouchard and Auger 2014).

**GYPSY MOTH**

European gypsy moth (*Lymantria dispar*) was introduced to eastern North America in 1869 and has since spread across most of the eastern deciduous forest, fluctuating to outbreak densities approximately every 6–10 years. Gypsy moth is a generalist early-spring feeder that can survive and reproduce on hundreds of deciduous and some coniferous hosts (Elkinton and Liebhold 1990).
Mortality of first instar gypsy moth (*Lymantria dispar*) caterpillars has been estimated between 0–84% (Elkinton and Liebhold 1990) and between 0–90% (Hunter 1993) on different hosts. Extensive research on gypsy moth population dynamics has shown that density-dependent mortality in late larval stages contributes to gypsy moth fluctuations; this mortality appears mostly due to mammalian predators, nuclear polyhedrosis virus (NPV) at high population density (Elkinton and Liebhold 1990), and since 1989, a fungal pathogen (Hunter and Elkinton 2000).

However, Elkinton and Liebhold (1990) also state that “changes in factors that affect survival of early instars may in certain years result in densities of late instar larvae or pupae that approach the satiation points of predators” and hence contribute to outbreak dynamics. One major source of variation in gypsy moth early-instar survival is the extent and success rate of first instar dispersal by passive ballooning. Adult female moths are flightless and lay their eggs close to their pupation site, often on tree trunks. Field studies indicate that almost all first instar larvae undergo an initial dispersal episode in order to settle on host foliage, and some can balloon several times if they do not encounter suitable food. Most first instar larvae move only a few hundred meters, while some can cover up to a few km (Capinera and Barbosa 1976). Field experiments in which larvae were released near small trees showed that less than 10% reached the foliage, suggesting that dispersal mortality is very high (Capinera and Barbosa 1976) and prompting further research into conditions under which larvae disperse, since these can play an important role in population dynamics.
First instar gypsy moth are susceptible to starvation if they cannot find suitable host leaves within 5-10 days depending on temperature (Hunter 1993), and are more likely to disperse when they only encounter unacceptable food or are denied food (Capinera and Barbosa 1976). Further studies showed that neonates are more likely to disperse from poor quality (Elkinton and Liebhold 1990) or phenologically unsynchronized hosts (Erelli and Elkinton 2000, Hunter and Elkinton 2000), and when weather is poor during this dispersal phase, the associated mortality increases (Hunter 1993). The window of opportunity for early instar feeding is thus linked to dispersal abilities and can be defined in both temporal and spatial terms (Hunter and Elkinton 2000). Indeed, thanks to its polyphagy, a neonate gypsy moth larva on a tree that has not yet leafed-out is often able to disperse to a different species that has. However, phenological mismatch (leaf-out too early or too late) often occurs over large enough spatial scales such that dispersing larvae cannot find suitable food. Foster et al. (2013) showed this occurred in 5 of 11 years studied. Their study also showed that, in an average year, 60% of their Appalachian landscape included host trees with suitable phenology for the gypsy moth, but that these stands occurred in different parts of the landscape in different years, and were often too far apart for larvae to disperse between them. Early-instar dispersal mortality thus depends on tree species and stand mix but also on spatial heterogeneity in tree phenology (Foster et al. 2013). Finally, larvae that hatch late relative to host plant phenology appear to experience lower predation rates later in larval development, such that phenological synchrony and dispersal early in development play a role in
population fluctuations via effects on late-instar mortality (Hunter and Elkinton 2000).

Similarly, pathogen-induced mortality in the late larval instars varies according to the species and phenology of foliage ingested with the virus, and this effect is correlated with levels of hydrolysable tannin – suggesting a tradeoff whereby plant defenses both hinder insect growth and protect the insect from disease (Cory and Hoover 2006). Recent work shows that late-emerging insects that are limited to feeding on mature foliage (which contains higher tannin levels) are more susceptible to NPV (Martemyanov et al. 2015) but less susceptible to B. thuringiensis infection (Martemyanov et al. 2016). The underlying mechanisms are not understood but could be related to effects on midgut biota: late-emerging larvae show dramatic decreases in the diversity of the midgut bacterial community (Martemyanov et al. 2016). The relationship between pathogens and foliar chemistry, notably phenology and induced defenses following defoliation, in their effects on gypsy moth outbreak dynamics remains difficult to establish (Cory and Hoover 2006).

Finally, it has been suggested that maternal effects increase dispersal rates of gypsy moth neonates (Rossiter 1991) as an adaptive response to decreasing stand quality following several years of defoliation during an outbreak. Dispersal occurs before feeding and therefore depends on the resources in the egg, acquired by the mother during her own larval stage (Diss et al. 1996). Leonard (Leonard 1970a, Leonard 1970b) found that smaller eggs from dense populations produce larvae with a longer pre-feeding period and a higher activity level, even in the presence of...
suitable food, and suggested that these larvae have a greater tendency to disperse. However, more recent work has failed to replicate this result (Diss et al. 1996), and suggests that the most important factors in dispersal-associated mortality are host-plant synchrony and weather during dispersal, not maternal quality or population density (Erelli and Elkinton 2000).

The last significant gypsy moth outbreak in North America occurred in 1981. In 1989, a population upsurge was checked by an accidentally introduced Asian fungal pathogen, aided by high rainfall in May and June of that year (Hajek et al. 1990). Outbreaks essentially ceased until 2015 when drought conditions in May and June prevented the fungus from controlling an upsurge that expanded into high defoliation rates in 2016 (U. Mass. Center for Agriculture, Food and the Environment). Whether this represents a climate-change induced regime shift remains to be seen.

**AUTUMNAL MOTH**

The autumnal moth (*Epirrita autumnata*) is a European generalist univoltine geometrid moth; destructive outbreaks occur with a 10-year cycle in northern and high-altitude populations of mountain birch (*Betula pubescens* ssp. Czerepanovii N. I. Orlova), whereas southern populations are more stable. This suggests that mortality sources are lagged density dependent in the North but directly density dependent in the South (Ruohomaki et al. 2000). Despite considerable research, it’s still not clear which mortality agents are responsible for these different dynamics, but the two main factors identified to potentially have density-dependent effects are natural enemies and plant defenses (Ruohomaki et al. 2000).
Natural enemies can cause very high mortality rates, up to 100% at some sites (Tanhuanpää et al. 2000, Klemola et al. 2014). All larval stages are parasitized, but not necessarily by the same species (Kaitaniemi and Ruohomäki 1999). A recent study shows that parasitism rates are highest in the egg and pupal stages, but also suggests that mortality in different populations and outbreak cycles can be dominated by not only different parasitoid species by different different guilds (egg vs larval vs pupal parasitoids) (Klemola et al. 2014). In general, larval parasitism shows strong lagged density dependence and predicts subsequent population growth rate (Tanhuanpää et al. 2000, Klemola et al. 2014).

Although natural enemy mortality is thought to be highest on late-instar larvae and pupae (Tanhuanpää et al. 2000, Klemola et al. 2014), plant traits clearly affect young larvae. In Northern outbreaking populations, autumnal moth larvae hatch at the time of budburst, and feed on the immature foliage of mountain birch (Ayres and MacLean 1987). The larvae can balloon on silk threads but do not disperse far, and dispersal loss is minimal (Tanhuanpää et al. 2000). Birch phytochemistry changes dramatically during leaf elongation (Riipi et al. 2002): flushing leaves contain high levels of flavonoid aglycones internally and on the leaf surface (Lahtinen 2004). As leaves mature, they become tougher, and water and Nitrogen content decrease, while hydrolysable tannins, flavonoid glycosides and later proanthocyanidins (condensed tannins) increase (Haukioja 2003). These changes delineate a complex window of opportunity for developing autumnal moth larvae.

At budbreak, the surface of birch leaves is covered with glandular trichomes that secrete exudates containing flavonoid aglycones, which become diluted rapidly as
leaves grow. The amounts vary considerably between individual trees but can reach up to 4% of initial leaf mass (Haukioja 2003). Mortality of first instar larvae is strongly correlated with these compounds and can reach 100% on hosts with high levels of surface flavonoid aglycones in expanding leaves (Lahtinen et al. 2004). However, neonate larvae can at least partially glycosylate these aglycones, rendering them more water soluble and easier to excrete (Lahtinen et al. 2006). Autumnal moth neonates thus possess physiological detoxification mechanisms that suggest an adaptation to feeding on young birch foliage, despite the apparent high mortality. Flavonoids are a complex group of compounds and the efficacy of first instar detoxification probably varies between compounds (Lahtinen et al. 2006). The amount and chemical structure of flavonoid defenses in flushing leaves could thus be an important predictor of larval survival.

Mature leaves not only contain high levels of condensed tannins (up to 15% of leaf dry mass), they are also very nutrient-poor, such that later-instar autumnal moth caterpillars need to consume very large amounts in order to acquire sufficient nutrients. The ratio of leaf consumption to larval growth increases from 2.9 in June, to 9.8 in early July, and to 15 in August (Haukioja 2003). It appears that young larvae are not able to achieve this rate of consumption on tough foliage or to process these high levels of condensed tannins. Indeed, early instar larvae grow faster on early season leaves (Ayres and MacLean 1987). Synchrony of larval hatching and leaf burst is essential for autumnal moth larvae, as early hatching neonates can starve in the absence of open buds and late hatchers seldom reach
pupation due to delayed development on poor quality foliage and high parasitism
later in the summer (Ruohomaki et al. 2000).

Rapid-induced resistance (chemical changes occurring in the same season as
defoliation; ie direct density dependence), delayed induced resistance (defensive
compounds that are produced in the year following heavy defoliation; ie indirect
density dependence) and indirect defences have long been hypothesized to play an
important role in generating autumnal moth outbreaks (Haukioja 2005). Notably,
birch defensive compounds can influence both insect growth and
immunocompetence, with potentially complex tri-trophic feedback involving
pathogens (Kapari et al. 2006). Recent molecular studies have proposed
mechanisms that could explain rapid-induced resistance (Ruuhola et al. 2008) and
delayed-induced resistance (Ossipov et al. 2014), and indirect defense via attracting
parasitoids (Klemola et al. 2012) and upregulating defences in neighbouring plants
(Giron-Calva et al. 2014). However, the importance of these mechanisms in
regulating field populations has been very difficult to demonstrate empirically,
probably in large part due to considerable inter-individual variation and rapid
phenological changes in mountain birch phytochemistry (Ruohomaki et al. 2000).
Although the ecological relevance of these processes has yet to be determined, they
nonetheless raise intriguing questions about the role of early-instar feeding in
triggering plant defences and increasing mortality in later stages or later
generations.

The *Epirrita* distribution range is expanding into colder, more continental climates,
creating novel disturbance regimes in these fragile subarctic ecosystems. Changes
in the temporal and spatial scales of *Epirrita* outbreaks, combined with a
concomitant range expansion of *O. brumata* into areas previously only affected by
*Epirrita*, is changing the dynamics of these mountain birch ecosystems in ways that
are not year clear. Research priorities formulated to address these challenges
include a better understanding of thermal and other mechanisms controlling
*Epirrita* egg hatch and birch budbreak in order to predict phenological relationships
(Ammunet et al 2015).

### MONARCH BUTTERFLY

The monarch butterfly (*Danaus plexippus*) is an icon for conservation biology.
Monarchs are best known for their extraordinary fall migration from across North
America to the mountains of Mexico where they overwinter in reproductive
diapause. In early spring, butterflies mate and fly north to the southern USA to lay
eggs on emerging milkweeds and die. The next generation migrates further north,
as does the next, until after 3-4 generations it is fall again and unmated butterflies
emerging in Canada begin the return migration. However, the geographic
distribution of *D. plexippus* is much broader: a separate population west of the
Rocky Mountains overwinters in California, and non-migratory populations are
found throughout Central America, South America and the Caribbean, as well as
more recently established populations in Oceania, Australia, Europe and Africa
(Zhan et al. 2014).

Monarchs are specialists on milkweeds, plants in the genus *Asclepias*, and feed on
at least 27 *Asclepias* species of the 100+ found in North America. Early instar
monarch larvae have variable but generally poor survival; average survival rates of
first instars vary between 3 to 40% depending on the host plant species (Zalucki et al 2012). Three traits—latex, cardenolides and trichomes—have been strongly implicated in milkweed resistance. These traits vary between species and decrease in shade-grown plants (Agrawal et al. 2012). When a milkweed leaf is cut, white viscous latex flows out and hardens upon contact with air. This latex contains cardenolides, steroids that target ATPase regulation and are toxic to animals. Production of cardenolides can also be induced following leaf damage. In some species, glandular trichomes offer additional mechanical defense and secrete toxic or sticky exudates. Each of these traits has been demonstrated to quantitatively affect the behavior, performance, and survival of early-instar monarch larvae in the field, despite the fact that these caterpillars are milkweed specialists and have adaptations to cope with them (Zalucki et al. 2012).

Indeed, first-instar monarch larvae exhibit specialised foraging behavior: once they have hatched from their egg and eaten part or all of the egg shell, they closely graze down trichomes to reach the surface and then carefully chew trenches through the epidermis to create a latex-free feeding zone (Agrawal 2012). Nonetheless, neonates can become mired in latex and permanently stuck when it hardens. Ingestion of latex can also cause larvae to become cataleptic and increase their chances of being mired, falling off the plant or being killed by invertebrate predators (Zalucki et al. 2001a). It is also at this early stage that caterpillars can acquire *Ophryocystis elektroscirrha* from the phylloplane: this neogregarine parasite causes developmental abnormalities during metamorphosis when ingested during the early instars (Leong et al. 1997).
A study examining defensive traits of 24 milkweed species found no trade-offs between defensive traits but instead clustered the species into three defensive strategies: one with low nutritional value, tough leaves and low water content (i.e., hard to eat, little reward), and two with more nutritious and edible leaves, but coupled with either high physical (trichomes, latex) or chemical (cardenolides) defenses. Survival of monarch larvae was similar on all three (Agrawal and Fishbein 2006), suggesting that a complex co-evolutionary arms race is operating in this plant–herbivore system.

Experiments in which larvae were reared on leaves with cut laticifers to reduce latex production showed different outcome on different milkweed species, linked to latex production and cardenolide concentration (Zalucki et al. 2001b, Zalucki et al. 2012). For instance, A. humistrata, one of the main hosts of the first generation of monarchs to reach the southern USA, contains high latex and high cardenolides. Cutting laticifers on this host improved survival (mortality due to miring in the latex was 27% on the intact leaves despite trenching behavior compared with 2% on the notched leaves), and doubled larval growth rate but decreased caterpillar body cardenolide content by half (Zalucki et al. 2001a). However, on the high-latex/low-cardenolide milkweed Asclepias syriaca, found in the northern USA and Canada, growth was more rapid on severed-laticifer leaves but survival was unaffected. Neither growth nor survival changed on the low-latex/low-cardenolide A. incarnata and A. tuberosa (Zalucki et al. 2001b).

Cardenolides consumed by monarch caterpillars are sequestered in body tissues where they appear to protect both larvae and adults from natural enemies and from...
disease (Malcolm 1995). On *A. syriaca*, a plant with low cardenolide content, larvae protected in cages had a higher survival rate (92%) than larvae left free on plants (24%) (Zalucki et al. 2001b). Adding cardenolides to artificial diet fed to monarch caterpillars infected with *O. elektroscirrha* slowed parasite growth and increased lifespan of infected but not uninfected butterflies (Gowler et al. 2015). Cardenolide consumption thus appears to mediate a trade-off between toxicity and protection. The production of these compounds is further influenced by yet another trophic level: arbuscular mycorrhizal fungi increase both cardenolides and latex production in infected plants, but the effect on monarch populations is not clear (Tao et al. 2016).

Thus, for neonate monarchs, the first bites into milkweed leaves are dangerous. However, later instars of this specialized herbivore appear better able to circumvent plant chemical and mechanical defenses because their larger mouthparts can more readily cut the laticifers and stop the latex flow (Zalucki et al. 2001b). Plant traits thus play a major role in early-instar survival (Zalucki et al. 2001b), but it is not clear how much this contributes to population dynamics or to the recent decline of North American migratory monarchs.

The main threats causing monarch population declines appear linked to the disappearance of milkweed plants linked to intensive agricultural practices especially in the USA corn belt (Flockhart et al. 2015, Stenoien 2016), although research also suggests declines occurring during migration or on overwintering grounds (Inamiine et al. 2016). Summer breeding success does not appear to be affected by changing climate (Stenoien 2016), but monarch populations could be
impacted by climate change via increases in extreme weather events during overwintering or reduced rainfall and concomitant decrease in milkweed abundance in the southern spring breeding grounds (Oberhauser et al. 2016). Conservation efforts must focus on the life history stages that affect population dynamics in order to be effective; for a long-ranging migratory species like the monarch, these can occur thousands of kilometers apart, across major biomes and jurisdictions, greatly increasing the challenges of conservation.

**CONCLUSIONS**

Life tables were constructed for many pest insects in the 1960s, often showing very high and very variable early instar mortality. Further research on several species has shown that neonate caterpillars are indeed very vulnerable, and their survival often depends on phenological relationships with both host plants and natural enemies. The strength, timing and density-dependence of these relationships will determine their effects on population dynamics, suggesting that one needs to consider specific individual-level mechanisms in their ecological context.

For instance, *C. fumiferana* and *C. pinus* share similar ecologies but different population dynamics linked to differences in early instar mortality (Nealis 2003). A previous section described how early-instar dispersal and mortality in *C. fumiferana* is tied to the availability of buds to emerging larvae, which in turn depends on host phenology and past defoliation.

The congeneric *C. pinus* shares a very similar ecology and life history, but very different population dynamics. It also hatches before budbreak of its main host,
jack pine, but does not mine old needles and hence depends strongly on presence of pollen cones for survival. Early instar survival is 60-80% when pollen cones are present on the host plant, but <10% when they are not. Pollen cone production decreases sharply in accordance with the severity of previous-year defoliation. Lack of pollen cones thus constitutes a lagged density-dependent mortality source which generates the short-lived outbreaks typical of *C. pinus* (Nealis 2003).

*C. fumiferana* also mines pollen cones, but, because it is also able to mine old foliage, the relationship between early-instar survival and previous defoliation is less strong. Thus, early-instar survivorship and its relationship to host phenology and previous defoliation drives short outbreaks in *C. pinus* and contributes to longer cycles in *C. fumiferana* (Nealis 2003). Explaining the difference in population dynamics between these two species requires an understanding of the relative strengths of the hostplant feedbacks affecting the survival of early instars.

Phenological relationships clearly play an important role in early-instar mortality rates, but their contribution to population dynamics is less predictable. For some species, like *C. pinus*, as well as *Operophtera brumata* and *Tortrix viridana*, synchrony with host plant seems to play an important role in generating outbreak cycles, via an effect on early-instar survivorship (Nealis 2003). For the four species examined here, the relationship is more complex. Counter-intuitively, the role of phenological relationships with host plants in early-instar natural enemy mortality has received little attention, but their effects on late-instar predation rates can be important (e.g. the well-documented slow-growth-high-mortality scenario or the reverse effect observed in gypsy moth (Hunter and Elkinton 2000)).
It is increasingly recognized that phenological tri-trophic interactions including host plant, herbivore, and natural enemies create emergent dynamics in herbivore population dynamics (Cooke et al. 2007, Stam et al. 2014). A particularly notable recent development is the realization that the third trophic level not only includes predators and parasitoids but also microbes (Cory and Hoover 2006). Notably, plant compounds that are deemed defensive because of their negative effects on insect performance have recently been shown in several systems to also boost insect immunity, with potentially complex tri-trophic feedback effects between plants, herbivores and pathogens (e.g. gypsy moth (Cory and Hoover 2006); autumnal moth (Kapari et al. 2006); monarch butterfly (Gowler et al 2015)). Phenology can drive these relationships in similar ways as with multicellular natural enemies, as insect immunity can vary with host plant synchrony (Martemyanov et al. 2015). The study of mycorrhizae (e.g Tao et al 2016), phylloplane flora (e.g. Leong et al. 1997), midgut biota (e.g. Mason and Raffa 2014, Martemyanov et al. 2016) and pathogens (e.g. Hajek et al. 1990, Leong et al. 1997, van Frankenhuyzen et al. 2007, Gowler et al 2015) in plant-herbivore systems is suggesting potentially important roles for multi-trophic interactions with these poorly-understood organisms. These relationships are only beginning to be identified and their phenologies or responses to climate have yet to be examined.

In this context, the current paper emphasizes the importance of considering the early larval instars, despite their small size and general intractability, because of the extremely high and variable mortality they generally experience and because of their particular sensitivity to phenology and hence to climate change.
ACKNOWLEDGMENTS

Thanks to Canada’s Natural Science and Engineering Research Council for funding, and to numerous students and colleagues for help with research underlying this work.

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