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OPTIMIZING MONARCH BUTTERFLY AND BEE CONSERVATION EFFORTS IN THE URBAN LANDSCAPE

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Dr. Kenneth Haynes, Director of Graduate Studies

OPTIMIZING MONARCH BUTTERFLY AND BEE CONSERVATION EFFORTS IN THE URBAN LANDSCAPE

DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Agriculture, Food and Environment
at the University of Kentucky

By
Adam M. Baker
Lexington, Kentucky
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2020

OPTIMIZING MONARCH BUTTERFLY AND BEE CONSERVATION EFFORTS IN THE URBAN LANDSCAPE

ABSTRACT OF DISSERTATION

The eastern migratory population of monarch butterflies (*Danaus plexippus*) is in serious decline. Most of the efforts to conserve this iconic insect focus on habitat restoration in the US Midwest. Often overlooked are small butterfly-centric gardens that can act as stepping stones between urban and rural areas. These studies aim to optimize the conservation value of such gardens.

Eight milkweed (*Asclepias*) species varying in height, form, and leaf shape were compared over two years in a common-garden experiment. I measured milkweed growth, rhizome spread, and bloom periods, conducted bi-weekly counts of monarch eggs and larvae, evaluated suitability for larvae, and quantified bee visitation. More monarchs were found on taller, broad-leaved milkweeds, but there was relatively little difference in larval performance. *Asclepias tuberosa* attracted the greatest number of bees, whereas bee genus diversity was greatest on *A. verticillata.*

Gardens containing the identical mix of milkweeds, flowering plants, and grasses but arranged in three different spatial configurations were monitored for monarch colonization over two years. Monarch eggs and larvae were 2.5–4 times more abundant in gardens having milkweeds evenly spaced around the perimeter than in gardens in which milkweeds were surrounded by or intermixed with the other plants. Predator populations were similar in all garden designs. In a corollary experiment, female monarchs laid significantly more eggs on plants that were fully accessible than on milkweeds surrounded by non-host grasses. In addition, I monitored monarch use in 22 citizen-planted gardens containing milkweed and nectar plants in relation to their botanical composition, layout, and surrounding hardscape. Significantly more monarchs were found in gardens having milkweeds spatially isolated and in gardens having 100 m north/south access unimpeded by structures.

The high-profile model system of milkweeds and monarchs was used to test if cultivars have equal conservation value as native wild-types. In replicated gardens I compared two species of milkweed (*A. incarnata* and *A. tuberosa*) and three of their cultivars over two years, measuring plant size, defensive characteristics, colonization by monarchs, suitability as host plants, and the bee assemblages, and Lepidopteran communities of each. I found that horticultural selection enhanced defensive characteristics in some cultivars, but did not influence larval growth and development. I also compared defensive characteristics of non-native milkweeds (*A. curassavica* and *Gomphocarpus physocarpus*) and their cultivars in the greenhouse and observed similar results.

The European paper wasp or EPW (*Polistes dominula*) predominantly builds its nests on structures. These invasive wasps forage for soft bodied arthropods, including monarch larvae, which may cause conservation gardens to become ecological traps. I confirmed EPW is the predominant *Polistes* spp. in urban gardens, documented outcomes between EPW and monarch larvae, and found that predation by EPW was more common in urban gardens than rural grasslands away from structures.

I found that the invasive Japanese beetle (*Popillia japonica*) aggregates and feeds on flowers of *A. syriaca*, the monarch's most important host plant, reducing seed set by >90%. The beetle's ongoing incursion into the monarch's key breeding grounds in the US Midwest is likely to limit pollination and outcrossing of wild and planted milkweeds, reducing their capacity to colonize new areas via seeds.

KEYWORDS: *Danaus plexippus*, pollinator conservation, urban gardens, *Asclepias, Polistes dominula, Popillia japonica*

Adam M. Baker

04/23/2020

Date

OPTIMIZING MONARCH BUTTERFLY AND BEE CONSERVATION EFFORTS IN THE URBAN LANDSCAPE

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04/23/2020
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DEDICATION

To my Mother and Father

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CHAPTER 1

Introduction

The eastern migratory population of the monarch (*Danaus plexippus*), a butterfly that performs a spectacular long-distance migration each year (Figure 1.1), has experienced severe decline in the last few decades (Brower et al. 2012; Rendon-Salinas et al. 2015). This decline has been attributed in part to an estimated 80% loss of milkweeds throughout the Midwestern United States, the monarch's most important summer breeding grounds, due to agricultural intensification and the spread of urbanization (Pleasants and Oberhauser 2013; Pleasants et al. 2017; Zaya et al. 2017). A census of overwintering butterflies in winter 2014 revealed the lowest-ever recorded density since monitoring efforts began (Rendon-Salinas et al. 2015). These reports prompted action to restore monarch habitat, including adding millions of milkweed plants, to the migratory flyways of North America (Pleasants et al. 2017; Thogmartin et al. 2017a). Although not all scientists are convinced that milkweed limitation is a major factor in monarch decline (Davis and Dyer 2015; Dyer and Forister 2016; Inamine et al. 2016; Agrawal 2017), restoration of milkweeds in many land-use types is already underway. Regardless of whether those actions will help to stem monarch butterfly decline, planting milkweeds can help share the evolutionary story of the monarchs and their toxic host plants with many enthusiastic gardeners and young naturalists.

Most of the effort to restore milkweeds has focused on reserve farmlands, roadsides, conservation easements, and other agriculturally dominated landscapes (Thogmartin et al. 2017b). The goal of increasing the carrying capacity of the Midwestern summer breeding grounds, where nearly 40% of all the monarchs that end up at the overwintering grounds come from (Flockhart et al. 2017), may be important for stabilizing the eastern monarch population. Often overlooked, however, are urban areas, where citizens are eager to incorporate butterfly gardens and other greenspace to support desirable wildlife, especially birds, bees, and butterflies (Goddard et al. 2010). But is monarch conservation in the urban landscape a good idea? Or are there glaring problems with this approach? There is still much that we don't know about the way insects perceive and interact with urban environments and the threats therein. With urban areas expanding, small urban and suburban gardens may be a valuable piece of the "all hands on deck" strategy to meet the existing goal of restoring 1.8 billion milkweed stems to the monarch's summer breeding range (Thogmartin et al. 2017b). This dissertation explores ways to increase the value of urban sector's contributions to monarch conservation.

Biology of the monarch

Monarch butterflies are renowned for their annual long-distance migration throughout North America to their overwintering grounds in central Mexico. Each year the monarchs, under reproductive diapause, spend the winter in about a dozen discrete locations in the Mexican highlands (Malcolm and Zalucki 1993). In spring they make their way to southern Texas and begin their breeding to coincide with the emergence of their host plants. Through subsequent generations, the monarchs will make their way to the summer breeding grounds in northern United States and even up into Canada (MonarchWatch 2019). When summoned by a suite of environmental cues (photoperiod, temperature, and host plant quality) the butterflies begin their journey back to the overwintering grounds (Malcolm and Zalucki 1993; Agrawal 2017).

Monarchs are host plant specialists that require milkweeds (Apocynaceae; Asclepiadoideae) (Agrawal and Fishbein 2006), or closely related species to complete their development (Bartholomew and Yeargan 2001; Yeargan and Allard 2005). Milkweeds are a diverse group that contain > 100 species (Figure 1.2) in North America (Woodson 1954) and 33 of those species have been reported to be utilized by monarchs. Milkweeds contain cardiac glycosides (Brower and Fink 1985; Agrawal and Fishbein 2006), toxic steroidal compounds known as cardenolides, which they use as a defense mechanism against herbivore attack. The monarch butterfly exploits these compounds and sequesters them in fatty tissues as a defense mechanism (Brower and Fink 1985). The eggs that are laid on milkweeds hatch, with larvae going through five developmental instars before leaving the plant to form a chrysalis (Malcolm and Zalucki 1993), the process from a neonate larva to adult taking about three weeks.

In order to feed on milkweed, monarchs must overcome the physical defense measures of the plant. When injured, milkweeds exude a sticky, viscous fluid called latex that can gum up the mouthparts of insects or even trap them (Zalucki et al. 2001). After hatching the neonate larvae trim surrounding trichomes and cut a trench in the leaf to subdue latex flow before feeding begins (Dussourd 1999). Late instar larvae may avoid latex by severing the veins of milkweed leaves, stopping the vascular flow so they can feed on the undefended leaf (Dussourd and Eisner 1987).

Host-finding

Host-finding by monarchs is influenced by plant size, age, leaf shape, isolation, defensive characteristics, and other factors. Height and size of plants can play a role in oviposition as monarchs that tend to prefer taller plants (Cohen and Brower 1982; Zalucki and Kitching 1982b). Cutting and Tallamy (2015) found that gardens in suburban areas had greater oviposition by monarchs than did similar plantings in natural areas. More eggs were found on a per-plant basis in garden settings, but survival was equal between the two locations (Cutting and Tallamy 2015). In similar studies, more monarch eggs and larvae were observed in lower density milkweed patches than high density patches (Zalucki and Kitching 1982ab; Zalucki and Suzuki 1987) and patches in open areas and along edges had greater colonization as opposed to patches within forest boundaries or amongst competing vegetation (Zalucki and Kitching 1982a; Cutting and Tallamy 2015). Planting milkweeds on the perimeter of the garden to increase apparency and accessibility can lead to greater oviposition by wild monarchs (Baker and Potter 2019). Similar trends have been observed in other visually oriented diurnal butterflies (e.g. swallowtails) in relation to their host plant *Aristolochia* spp. (Rausher 1981). Long-winged butterflies (Heliconidae) use search imaging and learning (Rausher 1978), which may also play a role in host plant recognition for monarch butterflies.

For monarchs, isolated plants have the greatest per-plant number of eggs and larvae than larger patches or clusters (Zalucki and Kitching 1982ab; Zalucki and Suzuki 1987). This has been observed in other specialist butterflies as well (Cromartie 1975; Jones 1977; Rausher 1981; Mackay and Singer 1982). Isolated plants, or plants spaced so that they are not interacting directly with other plants, may be more apparent (Feeny 1976) and easier to locate by female monarchs.

Host acceptance by monarchs has been suggested to be influenced by compounds such as flavanol glycosides (Haribal and Renwick 1998), cardenolide content (Zalucki et al. 1990), and quality of the foliage. When a female monarch encounters a host plant the path to acceptance is driven by sensory organs located on the antennae, forelegs, and midlegs (Haribal and Renwick 1998). Not all sensory organs are used equally on different milkweed species. For instance, when monarchs encounter swamp milkweed (*A. incarnata*) they use their forelegs, on tropical milkweed (*A. curassavica*) they use antennae, and on butterfly weed (*A. tuberosa*) they use all three appendages (Haribal and Renwick 1998).

Brief history of monarch conservation

Numerous conservation programs, with the help of citizen scientists, have joined together in efforts to save this beloved butterfly and preserve the great migratory phenomenon. The conservation of the monarch butterfly is valued in the billions of dollars, amounts similar to those of endangered vertebrate animals (Diffendorfer et al. 2014), which is unprecedented for any arthropod. The first monarch citizen science effort, led by Fred and Norah Urquhart, recruited thousands of volunteers to report sightings of south-bound butterflies. The project was ultimately a success with the discovery of the monarch overwintering grounds and was featured on the cover of National Geographic in August, 1976, the photo depicting a citizen scientist amongst the butterfly –littered forests of central Mexico. This was just the start of the citizen science movements surrounding the charismatic monarch butterfly. Each year thousands of volunteers participate in monarch garden establishment, tagging/monitoring of butterflies, and attend educational series involving monarchs and other pollinators (MonarchWatch 2020, Journey North 2017, MLMP 2019, Project Monarch Health 2019).

Many conservation organizations and programs throughout North America have stepped up to the plate to combat monarch population decline (Table 1.1). The Monarch Waystation Program, started in the mid 90's by MonarchWatch, recognizes participants on a national registry for installing monarch conservation gardens. Since its initiation the program has amassed 27,529 Waystations (Monarch Watch 2020). The Million Pollinator Garden Challenge, a program very similar to Monarch Waystation was spurred from the Pollinator Protection Health Task Force (PHTF 2015) mandate. Since the initiation in 2015, their goal has been met in just three short years (MPGC 2020) amassing > 1 million gardens, many of which are likely to contain milkweed. Although the monarch butterfly is not in itself a prolific pollinator, it has become a poster insect for pollinator conservation. Programs like these offer opportunities for actionable science by participants in urban areas and can help urban residents reestablish their connection with nature. Whether or not small butterfly-centric gardens contribute to the ecological success of the monarch the educational and therapeutic value of such programs is undeniable.

Organization of this Dissertation

This dissertation consists of a General Introduction (Chapter 1), five primary research chapters, and Summary and Implications (Chapter 7). At the time this is written, three of five research chapters have been published in refereed scientific journals and the remaining two are close to submission. All of this work explores the conservation of the monarch butterfly with an emphasis on habitat in urban areas.

Chapter 2 (published; *Journal of Insect Conservation 22:405–418; 2018*) evaluates eight species of milkweed for growth characteristics, suitability as food for monarch larvae, and colonization and use in the field by monarchs and bees. Chapter 3 (published; *Frontiers in Ecology and Evolution Vol. 7; article 474*) explores how the location and layout small urban gardens affect use by monarchs. In Chapter 4 (near submission to *Peer J*), I use the milkweed system to test the hypothesis that cultivars of native plants can be as suitable as wild-type milkweeds for monarch butterflies and bees. Chapter 5 (near submission to *Scientific Reports*) documents the counterpoint that predation by an invasive paper wasp can turn urban gardens into ecological traps for monarch larvae. Chapter 6 (published; *Scientific Reports Vol. 8; article 12139*) concerns Japanese beetle (*Popillia japonica*) florivory on common milkweed (*A. syriaca*) and implications for milkweed restoration.

I sometimes use the plural words "we" and "our" when describing methods, observations, and results in the primary research chapters because I was often assisted by other lab members (especially undergraduate summer helpers) when setting up and evaluating trials, and by my Major Professor when planning experiments. Nevertheless, I was the primary hands-on investigator for all of the research described herein.

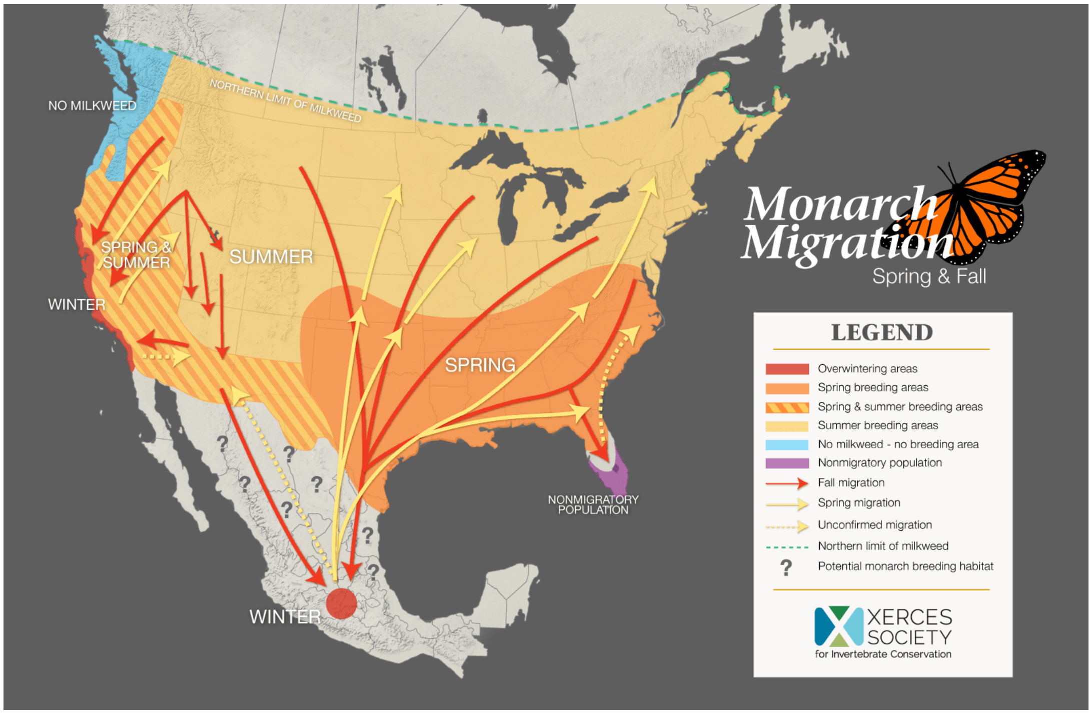

Figure 1.1 North American monarch migration routes. Source: Xerces Society

A. fascicularis *A. incarnata* *A. latifolia* *A. speciosa*

A. syriaca *A. tuberosa* *A. verticillata* *A. viridis*

Figure 1.2 Examples of different milkweed *Asclepias* spp. These species were utilized in the urban garden research described in Chapter 2. Photographs taken by author unless otherwise noted.

Table 1.1 Organizations and programs involved in monarch butterfly conservation

Organization/Project	Location	Role
Audubon International	United States	Golf courses
Balcones Canyonlands National Wildlife Refuge	TX	BMPs for habitat restoration
BASF	United States	Agricultural lands
Board of Trustees of the University of Illinois	IL	Roadsides and utility rights-of-ways
Compatible Lands Foundation	TX	US Military bases
Correo Real	Mexico	Monitoring
David Suzuki Foundation: One Nature	International	Research funding
Department of Natural Resources	MI	Public and private lands
Environmental Defense Fund	TX	Private lands
Field Museum of Natural History	IL, IN, IA, MN, MO, WI	Urban areas
Houston Wilderness	TX	Urban areas
Iowa Natural Heritage Foundation	IA	Roadsides/Flyways
Iowa State University	IA	Reserve farmlands/education
Journey North	United States	Monitoring/education
Kentucky Transportation Cabinet	KY	Roadsides/gardens at rest areas
La Cruz Habitat Protection Project	Mexico	Overwintering habitat reforestation
Make Way for Monarchs	United States	Milkweed protection/education
Midwest Association of Fish and Wildlife Agencies	Central US	Track and implement new strategies
Missouri Conservation Heritage Foundation	MO	Rural and urban areas
Monarch Joint Venture	United States	Education/roadsides/flyways
Monarch Watch	US and Mexico	Education/gardens/monitoring/tagging
National Pollinator Garden Network	International	Gardens/education
National Wildlife Federation	United States	Urban/policy
Natural Partners	United States	Education
Nature Conservancy of Canada	Canada	Agricultural lands
Nebraska Game and Parks Commission	NE	Prairie enhancement and restoration
New Jersey Audubon	NJ	Monitoring
North American Butterfly Association	United States	Monitoring
Partners of Fish and Wildlife Program	IA, MN, ND,TX	Public and private lands
Peninsula Point Monarch Research Project	MI	Monitoring
Pheasants Forever	IL, IN, IA, MN, MO, OK, TX, WI	Private lands
Pollinator Health Task Force	United States	Policy
Prairie Pothole Partners	ND	Agricultural lands
Regents of the University of Minnesota	United States	Monitoring
Southwest Monarch Study	AZ	Tagging/monitoring

St. Louis Municipal Government	MO	Gardens
St. Marks National Wildlife Refuge	FL	Tagging/monitoring
Syngenta	United States	Golf courses
Toronto and Region Conservation Authority	Canada	Education
United States Division of Agriculture	United States	Policy/strategy development
US Fish and Wildlife Service	United States	Policy/planning/education/partnerships
University of Georgia	GA	Disease monitoring
Ventana Wildlife Society	CA	Monitoring
Wetlands Initiative	IL	Prairie and wetland restoration
Wild Ones	United States	Education/leadership
Wisconsin Department of Natural Resources	WI	Mississippi river corridor
Xerces Society	United States	Education/habitat restoration

CHAPTER 2

Colonization and usage of eight milkweed (*Asclepias*) species by monarch butterflies and bees in urban garden settings

Introduction

Monarch butterflies (*Danaus plexippus* L.) migrate annually from overwintering sites in the oyamel fir forests of central Mexico to broad regions across North America, east of the Rocky Mountains, a migratory cycle typically requiring four generations (Malcolm and Zalucki 1993; Agrawal 2017). Monarch larvae feed exclusively on milkweeds (family Apocynaceae, subfamily Asclepiadoideae), including true milkweeds in the genus *Asclepias* (Agrawal and Fishbein 2006) and their close relatives; e.g., *Cynanchum laeve* (Bartholomew and Yeargan 2001; Yeargan and Allard 2005). The eastern migratory population of monarchs has declined by > 80% since systematic censuses of numbers of overwintering adults began in the 1990s, falling to the lowest level ever recorded in winter 2013–2014 (Brower et al. 2012; Rendon-Salinas et al. 2015). Concerns about its long term viability have mobilized scientists, federal and state agencies, non-governmental organizations, and private citizens into actions to safeguard and restore monarch populations (Pollinator Health Task Force 2015; Gustafsson et al. 2015; Monarch Joint Venture 2018).

Although surveys suggest that milkweed populations have been relatively stable in more natural and semi-natural areas (Hartzler 2010; Pleasants and Oberhauser 2013; Zaya et al. 2017), when croplands and loss of natural habitat to urbanization are considered, there has been substantial loss of milkweeds in the monarch flyways

(Pleasants et al. 2017b; Zaya et al. 2017). Despite some scientists' questioning of a causal link between milkweed loss and monarch decline (Davis and Dyer 2015; Dyer and Forister 2016; Inamine et al. 2016; Agrawal 2017), the milkweed-limitation hypothesis has gained traction because it suggests a plausible strategy by which diverse stakeholders can work together in actionable science (Palmer 2012; Gustafsson et al. 2015) to help conserve the monarch and its migration. Planting milkweeds on public and private lands has emerged as a central conservation strategy (Thogmartin et al. 2017b; Monarch Joint Venture 2018; National Pollinator Garden Network 2018; US Fish and Wildlife Services 2018).

Public interest in monarch conservation is reflected in the more than 18,600 Monarch Waystation habitats (managed gardens containing milkweeds and nectar plants) that have been registered with Monarch Watch as of January 2018 (MonarchWatch 2018), and the countless other similar gardens that have been planted in residential landscapes, at schools, businesses, parks, zoos, golf courses, nature centers, and other public and private places. Irrespective of the ecological value for monarch populations, pollinator gardening provides opportunities to engage large numbers of citizens in reconciliation ecology (Rosensweig 2003a; Colding et al. 2006; Lundholm and Richardson 2010), which in turn can foster a deeper interest in nature conservation (Miller 2005; Goddard et al. 2010; Bellamy et al. 2017).

Natural stands of milkweeds are generally scarce in residential areas (Cutting and Tallamy 2015), so it seems intuitive that planting milkweed in urban or suburban butterfly gardens will attract monarch adults to oviposit. That assumption, which previously was supported mainly by observational data, was validated in experiments that compared monarch colonization and survival in small plots of common milkweed, *Asclepias syriaca*, planted in managed landscapes in residential neighborhoods and equivalent plots planted in minimally managed native meadows (Cutting and Tallamy 2015). In that study, oviposition was significantly higher on plants in residential settings than in natural areas, with no difference in subadult survival between the two types of habitats.

Milkweed species vary in growth form, height, leaf shape and size, floral morphology and bloom time, and extent to which they spread vegetatively via rhizomes (Woodson 1954; Borders and Lee-Mäder 2015), so some species may be better suited than others for use in garden-type settings. *Asclepias syriaca* is the most important host for monarchs in their summer breeding range within eastern North America (Malcolm and Zalucki 1993; Flockhart et al. 2013, 2015), and nearly all habitat restoration models and recommendations are based on that species (Thogmartin et al. 2017b; Pleasants et al. 2017a). However, because of its height (up to 2 m) and propensity to spread, *A. syriaca* may be horticulturally less suitable than some other native milkweeds for managed gardens that, in addition to supporting monarchs, are designed to be aesthetically attractive while also providing resources for other pollinators.

Previous studies have examined monarch oviposition preference and larval performance in relation to defensive characteristics of the host plant (Agrawal et al. 2015), closely related species (Yeargan and Allard 2005), and larval growth on excised leaves, and on young plants in a greenhouse (Pocius et al. 2017a,b). Monarch oviposition is influenced by the height, age, and condition of milkweed plants, as well as their spatial dispersion and other factors (Zalucki and Kitching 1982a; Cohen and Brower 1982), so usage of different milkweeds in garden settings can not necessarily be inferred from laboratory or greenhouse trials. To date, no studies have compared monarch colonization and performance on different milkweed species in a replicated, common garden experiment in the field.

Gardening for pollinators is promoted by prominent conservation organizations (National Pollinator Garden Network 2018; National Wildlife Federation 2018; Pollinator Partnership 2018), and many gardeners are interested in growing plants that attract bees as well as butterflies (Garbuzov and Ratnieks 2014a,b). With wild bee populations declining in North America and globally due to agricultural intensification and loss and degradation of natural habitats (Beismeijer et al. 2006; Koh et al. 2016; Potts et al. 2016), urban butterfly gardens can play a role in supporting wild bee biodiversity (Hernandez et al. 2009; Baldock et al. 2015; Hall et al. 2017). Milkweed flowers produce abundant nectar and are highly attractive to bees (Borders and Lee-Mäder 2015). Research on bee visitation to milkweeds has focused mainly on determining which types of floral visitors are most effective at extracting and transferring pollinia (Kephart 1983; Betz et al. 1994; MacIvor et al. 2017), as opposed to documenting different milkweed species' relative attractiveness to bees or differences in the bee assemblages that visit them as a nectar resource in garden settings. Planting milkweed that attract and sustain bees as well as monarchs could boost the conservation value of gardens at no additional cost.

In this paper, we describe a two-year study comparing suitability of eight species of milkweed for such use in managed gardens. We assessed colonization and usage by wild monarchs over two growing seasons, compared larval performance, and documented abundance of other milkweed specialist insect herbivores. The milkweeds' extent of tillering, growth characteristics, and bloom periods were evaluated. Finally, we assessed visitation by bees, and composition of bee assemblages associated with six of the eight milkweed species.

Materials and methods

Milkweed characteristics, monarch use, and other herbivores in replicated gardens

Eight species of milkweed (*Asclepias* spp.) were selected for evaluation based on their suitability for use in low-maintenance sites in full sun with limited supplemental irrigation. Five of the milkweeds, *A. syriaca* L. (common), *A. incarnata* L. (swamp), *A. tuberosa* L. (butterfly), *A. viridis* Walter (green, spider, or antelopehorn), and *A. verticillata* L. (whorled), are native to Kentucky, whereas the other three, *A. speciosa* Torr. (showy), *A. fascicularis* Decne. (narrow-leaf), and *A. latifolia* (Torr.) Raf. (broad-leaf), are native to the central or western United States (Woodson 1954; Borders and Lee-Mäder 2015). Seed was purchased from Prairie Moon Nursery, Winona MN, and planted in tree pots (3.8 cm diameter, 20 cm deep; Stuewe and Sons, Tangent, OR) containing commercial potting medium (Promix BX, Premier Tech Horticulture, Quakertown, PA) in late February. The seedlings were grown in a greenhouse, fertilized (Osmocote 5-9-12, Scotts, Marysville, OH), and transplanted to replicated garden plots on 16 May 2016, 1 week after the 90% probability of last frost date for Lexington, KY (National Oceanic and Atmospheric Administration 2018).

The main study was conducted at University of Kentucky Arboretum and State Botanical Garden of Kentucky, Lexington, KY (GPS coordinates: 38.0139, -84.5052). This arboretum was an ideal site for this research because it reflects a typical residential setting consisting of a mixture of ornamental trees, shrubs, and gardens surrounded by low-maintenance turfgrass lawns located within a medium-sized city. Five milkweed gardens, each 1.22 × 9.75 m, were tilled and covered with landscape fabric. We subdivided each garden into eight plots (1.22 × 1.22 m), one for each of the milkweed species which were arranged in a randomized complete block. Four seedlings were planted 0.6 m apart within each plot. Height of the seedlings ranged from 16–30 cm at planting. The gardens were covered with shredded hardwood mulch (5 cm depth) and watered to aid plant establishment. The gardens were oriented in an east-west direction and separated from one another by at least 20 m.

We conducted counts of monarch eggs and larvae on all plants in the gardens once every two weeks from May to October 2016 and from April to September 2017. In addition, plants were measured for height, bloom presence, and colonization by milkweed specialist herbivores including *Aphis nerii* (oleander aphid), *Oncopeltus fasciatus* (large milkweed bug), and *Tetraopes* spp. (milkweed longhorn beetles) in July and August during each of the growing seasons. For aphids, each plant was rated by two independent observers for the overall percentage of plant that was infested on a 1–5 scale with (0 = no infestation, 1 = < 20%, 2 = 21–40%, 3 = 41–60%, 4 = 61–75%, 5 = >75%) for. For the other herbivores, actual numbers were counted. In 2017, tiller production was recorded by counting ramets that had escaped from the original garden plots. Those counts were taken in September near the end of the growing season.

Performance of monarch larvae on milkweed

Monarch larval growth and survival on the different milkweed species was compared in two field trials (plots as described above) and a greenhouse trial. Cohorts of larvae (mostly late first instars, some early second instars) were purchased from Idlewild Butterfly Farm (Louisville, KY) for each field trial. For Field Trial 1, the larvae were caged in fine mesh white bags (25 × 40 cm) on two plants per plot, with one bag per plant and two larvae per bag, using a similar proportion of first and second instars for each plot. The larvae were placed on the plants on 19 August 2016 and left to feed for 9 d, after which we recorded final weight, instar, and survival. By the start of the second field trial (15 September 2016), some plants had begun to senesce. We therefore caged larvae on nine healthy plants of each species distributed across the gardens, using three larvae per bag, and analyzed that trial as a completely randomized design with plants as replicates. Larval performance was evaluated after 7 d. Other procedures were the same as for Field Trial 1.

A third trial, conducted in 2017, compared larval growth on the aforementioned milkweed species in the greenhouse under standardized conditions, i.e., without possible variation in shading from neighboring plants, soil moisture, or other factors that might influence plant quality or larval performance in the field. Procedures for growing the milkweeds were as described for the replicated garden study, except that larger (10.1 cm diameter, 36 cm deep) pots were used. The seeds were planted in May 2017, and resulting plants were inoculated with first instar larvae on 18–23 August, by which time the milkweeds were 30–50 cm tall, depending on species. Each plant received a single neonate (< 1 d old) caterpillar confined in a fine mesh bag (25 × 40 cm) that covered most of the plant. *Asclepias viridis* seeds planted for this assay failed to germinate, so that species was dropped from the trial. There were 10 plants (replicates) each of seven milkweed species in a randomized complete block on the greenhouse benches. Plants were rotated twice a week on greenhouse benches to reduce site variation. Larvae were allowed to feed for 5 d, after which we assessed their instar and weight. Greenhouse temperatures while the larvae were on the plants ranged from 26 –28 ºC.

Bee assemblages on milkweeds in gardens and at other field sites

Bee assemblages visiting the different milkweed species in the replicated gardens were assessed in 2017. Relative attractiveness was compared by 2-min "snapshot" counts (Garbuzov and Ratnieks 2014a) taken twice at each plot during peak bloom (June to early July). Snapshot counts were taken on clear warm days (temperature > 20 °C, wind < 20 km/h), with one count in late morning (1100 −1200 h) and another in afternoon (1400−1600 h). At each visit, we counted the number of bees actively foraging on blooms of the milkweed plants in a given plot, trying not to count individuals more than once. Counts from the two visits were averaged and plants were assigned a rating where < 5 = low, 5–10 = moderate, and >10 bees = high, in addition to the mean count per plot.

After snapshot counts were completed, we collected a 30-bee sample from milkweed flowers in each plot (150 bees from each milkweed species that bloomed sufficiently). Sampling involved walking from plot to plot during mid-day (1100–1600 h) and knocking the first 30 bees observed on open flowers into plastic containers partially filled with 75% ethanol. Sampling of most of the milkweed species was completed over 1–3 successive days, depending on extent of bloom. Two of the milkweed species, *A.*

viridis and *A. latifolia*, did not bloom sufficiently for such a sample to be possible. The bees were cleaned and prepared for identification according to guidelines in Droege (2015), pinned, and identified to genus using online keys (Packer et al. 2007). Honey bees (*Apis mellifera* L.), bumble bees (*Bombus* spp.), and carpenter bees (*Xylocopa* spp.) were identified to species (Colla et al. 2011).

To assess if bee assemblages visiting milkweeds in the replicated gardens were representative of those associated with milkweeds at other central Kentucky field sites, we collected additional 50-bee samples from natural stands or plantings of *A. incarnata* (five sites), *A. syriaca* (four sites), and *A. tuberosa* (five sites) in parks, golf course naturalized roughs, butterfly gardens, and other locations in or near Lexington. Those samples, collected during peak bloom (16 June to 5 July) in 2016 or 2017, were prepared and identified as described above.

Data analyses

Plant characteristics (tillers, height) and insect abundance in the main garden study were compared among milkweed species by two-way analysis of variance (ANOVA) for a randomized complete block design with mean separation by Fisher's least significant difference (LSD) test when the overall treatment effect was significant ($P < 0.05$). Single degree of freedom contrasts were used to further compare monarch abundance between selected sets of milkweeds, e.g., tall versus shorter species, and narrow-leaved versus broad-leaved ones. Log- or square root- transformations were applied in cases where raw data failed to meet the assumptions of parametric statistical tests for normality or homogeneity of variance. For the field trials comparing monarch larval performance between milkweed species were similarly analyzed, except that for Field Trial, we used a completely randomized design with individual plants as replicates.

Chi-square tests for heterogeneity were used to test for differences in proportional representation of different bee taxa in collections from different milkweed species. Bee genus richness and diversity (Simpson 1-D; Magurran 2004) were compared between milkweed species by ANOVA for a randomized complete block (garden data) or completely randomized designs (data from sites other than the replicated gardens). Statistical analyses were performed with Statistix 10 (Analytical Software 2013). Data are reported as original (non-transformed) means ± standard error (SE).

Results

Milkweed characteristics in gardens

The eight milkweed species differed in height, form, and propensity to spread via rhizomes (Table 2.1). *Asclepias fascicularis*, in particular, produced numerous tillers. *Ascelpias verticillata* and *A. speciosa* also spread via rhizomes, the latter spreading several meters beyond the plot borders. The other milkweed species produced relatively few or no tillers. The milkweeds also varied in height (Table 2.1), with the taller species (*A. syriaca*, *A. speciosa*, *A. incarnata*, and *A. fascicularis*) attaining 1–1.7 m height by the second growing season after transplanting. All but three of the species bloomed in 2016 (*A. syriaca, A. speciose, A. latifolia*), and all eight bloomed in the following year. Bloom periods varied from May to August, and the different species varied in their attractiveness to bees (see below).

Monarch usage and abundance of other herbivores on milkweeds in gardens

All of the gardens attracted monarchs, with eggs and larvae found throughout the 2016 and 2017 growing seasons (Fig. 2.1). In 2016, the first monarch progeny were found in May, within a few weeks after the seedlings had been transplanted. In that first year, colonization of the gardens peaked in July and persisted until October, even after the plants had begun to senesce. Warm weather and strong northerly winds were associated with unusually early northward migration of monarchs in 2017 (Journey North 2017) which was reflected in high numbers of monarchs found in our gardens in April (Fig. 2.1). Usage by monarchs continued throughout the summer, peaking in August. No eggs or larvae were found past mid-September, reflecting the earlier senescence of the plants in 2017 compared to in 2016.

Numbers of monarch progeny found in the garden plots differed significantly between milkweed species in both years (Table 2.2). The taller species (*A. incarnata*, *A. syriaca*, *A. speciosa*, and *A. fascicularis*) recruited more monarchs than did the four shorter ones (t = 9.9, 8.6 for 2016 and 2017, respectively; $P < 0.001$; single degree of freedom contrasts). Milkweeds that were both tall and broad-leaved (*A. syriaca* and *A. speciosa*) were colonized more than all other species as a group (t = 6.9, 6.4 for 2016 and 2017, respectively; $P < 0.001$). In 2016, when *A. incarnata*, *A. syriaca*, and *A. speciosa* were of similar height (Table 2.1), more eggs and larvae were observed on *A. incarnata*, but the following summer, when *A. syriaca* and *A. speciosa* were taller than *A. incarnata*, more eggs and larvae were found on the former two species (Table 2.2). Compared to 2016, *A. tuberosa* recruited relatively more eggs and larvae in 2017, possibly reflecting their similar size to the other milkweed species during the monarchs' early arrival in April

2017. In total, we found 474 naturally-occurring monarch eggs and larvae on milkweeds in the gardens over the two growing seasons.

Aphid (*A. nerii*) populations also differed significantly among milkweed species (Table 2. 2). *Asclepias incarnata* and *A. latifolia* supported relatively high infestations of aphids in both years, whereas *A. fascicularis* had relatively few. On *A. incarnata*, which has relatively narrow leaves, most of the aphids were on stems and petioles. Aphids on *A. latifolia*, which has broad leaves, were mainly on abaxial leaf surfaces. Large milkweed bugs (*Oncopeltus fasciatus* (Dallas)) were found on all milkweed species but were particularly abundant on *A. syriaca*, *A. tuberosa*, and *A. fascicularis* which had pods throughout much of the growing season. Milkweed longhorn beetles (*Tetraopes* spp.) tended to be found mostly on *A. speciosa* and *A. fascicularis* (Table 2.2).

Performance of monarch larvae on milkweeds

Monarch larvae survived and developed on all milkweed species (Table 2.3). In Field trial 1, there was no difference in survival, but the final weight and instar attained differed significantly between the milkweed species, with relatively stronger performance on *A. verticillata*, *A. tuberosa*, and *A. speciosa*, and poorer performance on *A. fascicularis*. The larvae survived and grew similarly on all milkweed species in the other two trials (Table 2.3).

Bee assemblages on milkweeds in gardens and at other field sites

Six of the eight species of milkweeds in our gardens had accessible blooms and attracted enough bees to compare their overall attractiveness via snapshot counts (Figure

2.2). By that measure, *A. tuberosa* and *A. fascicularis* were particularly attractive to bees, followed by *A. syriaca*, *A. verticillata*, and *A. incarnata*. *Asclepias speciosa* attracted relatively few bees. Five families of bees were collected from milkweeds in our gardens (Table 2.4). The number of bee genera collected from particular milkweed species ranged from six on *A. speciosa*, to 13 on *A. verticillata* (Table 2.4). Bee genus richness (Simpson 1-D) differed significantly between milkweed species ($F_{5,15} = 2.93$; $P < 0.05$) and was significantly higher for *A. tubersosa*, *A. verticillata*, and *A. fascicularis* than for common milkweed, *A. syriaca*. The brown-belted bumble bee *Bombus griseocollis*, a common native species, and *Apis mellifera*, the European or western honey bee, were the most abundant bees sampled from milkweeds in our garden plots. Proportions of bees belonging to different taxa (*A. mellifera*, *Bombus* spp., *Xylocopa virginica*, Megachilidae, Halictidae, and combined other groups) differed significantly among milkweed species (Chi-square test for homogeneity; $\chi^2 = 316$, df = 25; Fig. 2.3). *Bombus* spp. dominated the bee assemblages visiting *A. syriaca*, *A. incarnata* and *A. tuberosa*. *Asclepias fascicularis* and *A. speciosa* were particularly attractive to *A. mellifera,* whereas *A. verticillata* attracted proportionately more Halictidae and other relatively small bees (Fig 2.4).

Assemblages of bees collected from *A. syriaca*, *A. incarnata* and *A. tuberosa* at the additional field sites were generally similar to those from the replicated gardens (Table 2.5). *Asclepias tuberosa* supported higher genus diversity than did either of the other two milkweeds ($F_{2,12} = 4.36$; $P < 0.05$; Table 2.5). Proportionate abundance of the different bee taxa differed significantly among those milkweed species ($\chi^2 = 104$, df = 10;

Fig. 2.3). *Bombus* spp. and *A. mellifera* dominated the samples from *A. syriaca* and *A. tuberosa*, whereas *A. incarnata* attracted a somewhat higher proportion of Halictidae.

Discussion

This study demonstrates that small urban gardens planted with milkweed are readily found and colonized by monarch butterflies. It supports the premise that planting Monarch Waystations (MonarchWatch 2018) or similar gardens is effective for augmenting monarch habitat in urban settings, and extends knowledge of how gardeners can best deploy milkweeds for conservation value. To our knowledge, this is the first study comparing usage of different milkweed species by monarchs and bees in a replicated outdoor common garden setting. Milkweeds in our gardens also recruited other specialist insects including aphids, milkweed bugs, and longhorn beetles. Although high densities of those herbivores can sometimes negatively affect seed production and become pests of milkweed crops (Borders and Lee-Mäder 2015), their presence in butterfly gardens is more likely to contribute interest and educational value.

Our gardens included eight milkweed species varying in height, growth form, leaf morphology, and propensity to spread by tillering (Borders and Lee-Mäder 2015; Lady Bird Johnson Wildflower Center 2018). All species were successfully established from transplants and regenerated in the second year, and all of them supported monarch larval growth and development. However, based on numbers of eggs and larvae found on the plants, they were not equally colonized by monarchs.

Host-finding and oviposition by monarchs are influenced by the height, age, developmental stage, and condition of milkweed in the field (Cohen and Brower 1982;

Zalucki and Kitching 1982a; Fischer et al. 2015). Females encountering single-species stands of milkweed in the field tend to lay more eggs on taller plants than on shorter ones (Cohen and Brower 1982, Zalucki and Kitching 1982a) which is consistent with our observations of more eggs and larvae on taller milkweed species (*A. syriaca, A. incarnata*, and *A. speciosa*) than on relatively shorter-statured ones in both years. Monarchs tend to lay more eggs per plant on isolated plants compared with milkweed in patches, and on plants on the edge of a patch as opposed to ones in a patch center (Zalucki and Kitching 1982a). Although the extent to which they use visual cues in host finding is unknown, other specialist butterflies (e.g., swallowtails, *Papilio* spp.) use search imaging to orient to host plants standing out against background vegetation (Rausher 1978, 1981). Short-statured milkweeds may go unnoticed by butterflies in mixed gardens because they are less apparent than taller milkweeds when surrounded by non-host plants. In a related study, milkweeds that were planted around the perimeter of small, mixed-plant gardens recruited more than twice as many monarchs as did same-sized milkweeds in the garden interior (Baker and Potter 2019). Female monarchs also tend to lay more eggs on younger plants (Zalucki and Kitching 1982a; Fischer et al. 2015), but all milkweeds in our gardens were of the same age.

Monarch eggs and larvae were first observed in our bi-weekly inspections in late May 2016, only two weeks after planting, indicating how rapidly the adults can find and utilize small gardens. At that time, the plants were < 30 cm tall. The milkweeds reached their maximum height by late July (Table 2.1), which in 2016 coincided with peak abundance of eggs and larvae. We continued to find sub-adult life stages in the gardens in September and October after many of the plants had begun to senesce. In 2017, the large number of eggs and larvae found in the gardens in April coincided with the inordinately early arrival of northward flying adults which was observed in many parts of the eastern flyway (Journey North 2017; Monarch Watch 2018).

All milkweed species in our study supported growth and development of monarch larvae. Others (e.g., Erickson 1973, Pocius et al. 2017 a,b) also found relatively little difference in performance of first instars reared on excised leaves of different milkweed species in the laboratory, or on young plants of those species in the greenhouse. The significant differences in abundance of wild eggs and larvae we observed on the milkweed species in our gardens probably reflect differential oviposition as opposed to host plant quality.

Milkweed flowers are long-lived, produce copious amounts of nectar (Wyatt and Broyles 1994), and are highly attractive to native bees, honey bees, butterflies, and other nectar-feeding insects (Fishbein and Venable 1996; Borders and Lee-Mäder 2015). Because milkweed pollen is enclosed within pollinia and is probably inaccessible as food, nectar is the only reward that milkweeds offer to their pollinators (Kephart 1983, Wyatt and Broyles 1994). Large bees in the family Apidae (honey bees, bumble bees, and carpenter bees), and some large wasps, moths, and butterflies are the most effective milkweed pollinators (Willson and Bertin 1979; Willson et al. 1979; Kephart 1983; Betz et al. 1994; Fishbein and Venable 1996; Ivey et al. 2003; MacIvor et al. 2017), whereas most of the smaller visitors are nectar thieves that do not provide pollination services to milkweed. Milkweeds, nevertheless, support a diversity of native bees that pollinate other cultivated and wild plants in urban habitats.

In our gardens, the bee assemblages of *A. syriaca*, *A. incarnata*, and *A. speciosa* were dominated by large apid bees. Large-bodied, eusocial bees have high energy demands (Heinrich 1976), so they may favor milkweeds having large flowers and profuse nectar rewards. Three other milkweeds, *A. tuberosa*, *A. verticillata*, and *A. fascicularis*, tended to attract proportionately more relatively small native bees. *Asclepias viridis*, has light green flowers and is among the first milkweeds to bloom in the Ohio Valley region (Taylor 2017).

Conclusions and Applications

Our findings will help gardeners and land managers to choose the milkweed species that best match their conservation goals. Milkweeds such as *A. incarnata* and *A. tuberosa* that "stay put" will integrate well with other plants in managed gardens. In contrast, tillering species such as *A. fascicularis, A. speciosa* and *A. syriaca* may be less well suited for managed gardens because of their tendency to spread into neighboring plant beds or lawns, but better for filling in larger land areas dedicated to monarch habitat restoration. Combining milkweed species that are preferred by egg-laying monarchs with ones such as that are particularly attractive to bees may be a strategy for increasing the conservation value of Monarch Waystations and similar small gardens. The location and spatial configuration of gardens may also influence discovery rates and colonization by monarchs. Small urban gardens containing milkweeds are readily found and colonized by monarch butterflies, so further research to determine how to optimize their value as part of a larger conservation strategy to save the monarch and its migration is warranted.

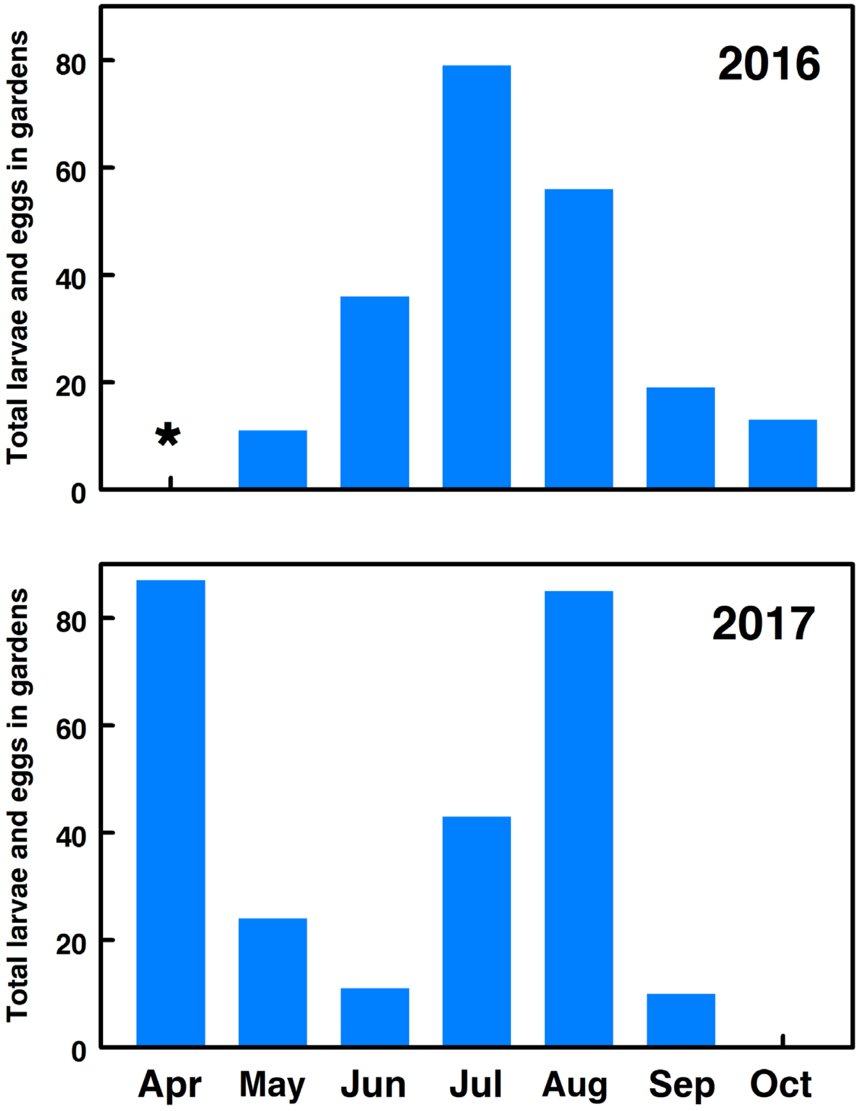

Figure 2.1 Seasonal abundance of naturally-occurring *Danaus plexippus* eggs and larvae on milkweeds in the experimental gardens in 2016 and 2017. Counts are totals across all eight milkweed species. *Young milkweeds were not transplanted until 16 May 2016, 1 week after the 90% probability frost-free date for Lexington, KY

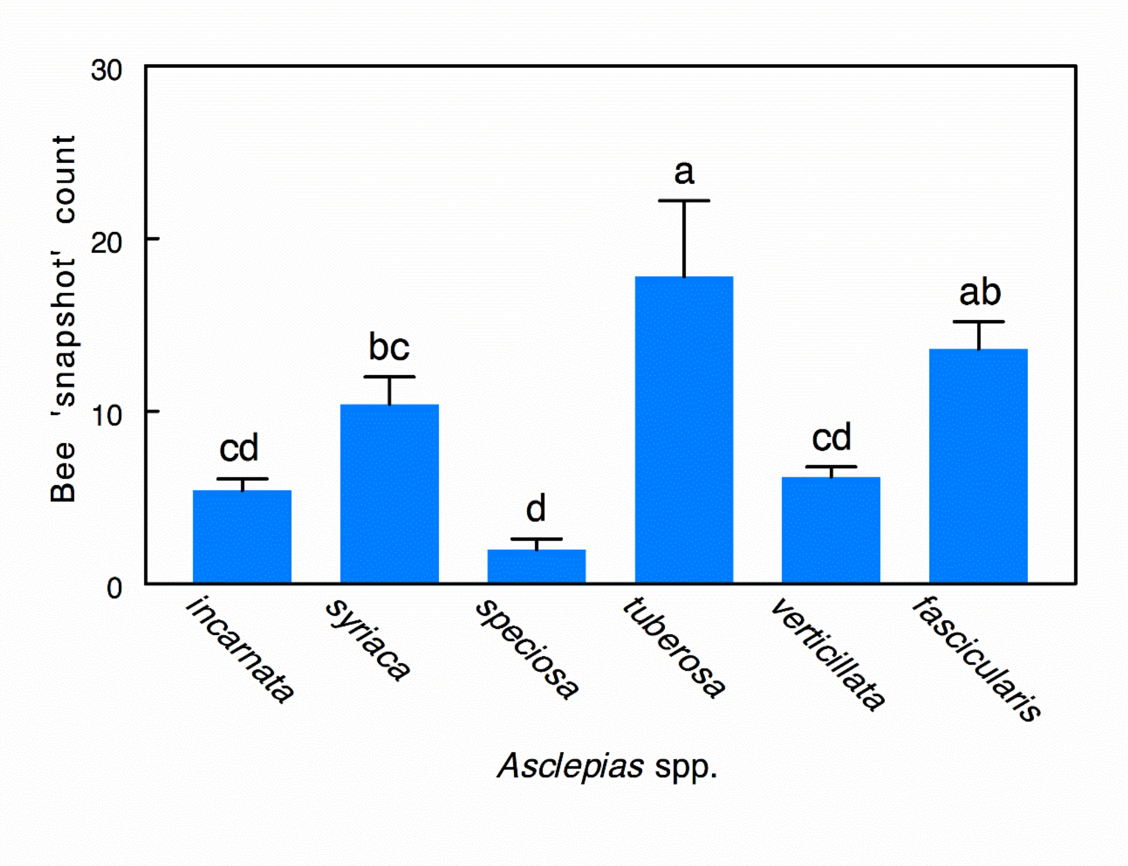

Figure 2.2 Relative attractiveness of different milkweeds to bees as measured by two 2-min “snapshot counts” in the late morning and mid-afternoon during each species’ peak bloom, 2017. Means (± SE) not topped by the same letter differ significantly ($F_{5,20} = 7.62$; LSD, $P < 0.005$). Snapshot counts were not taken for *A. viridis* and *A. latifolia* because they did not sufficiently bloom

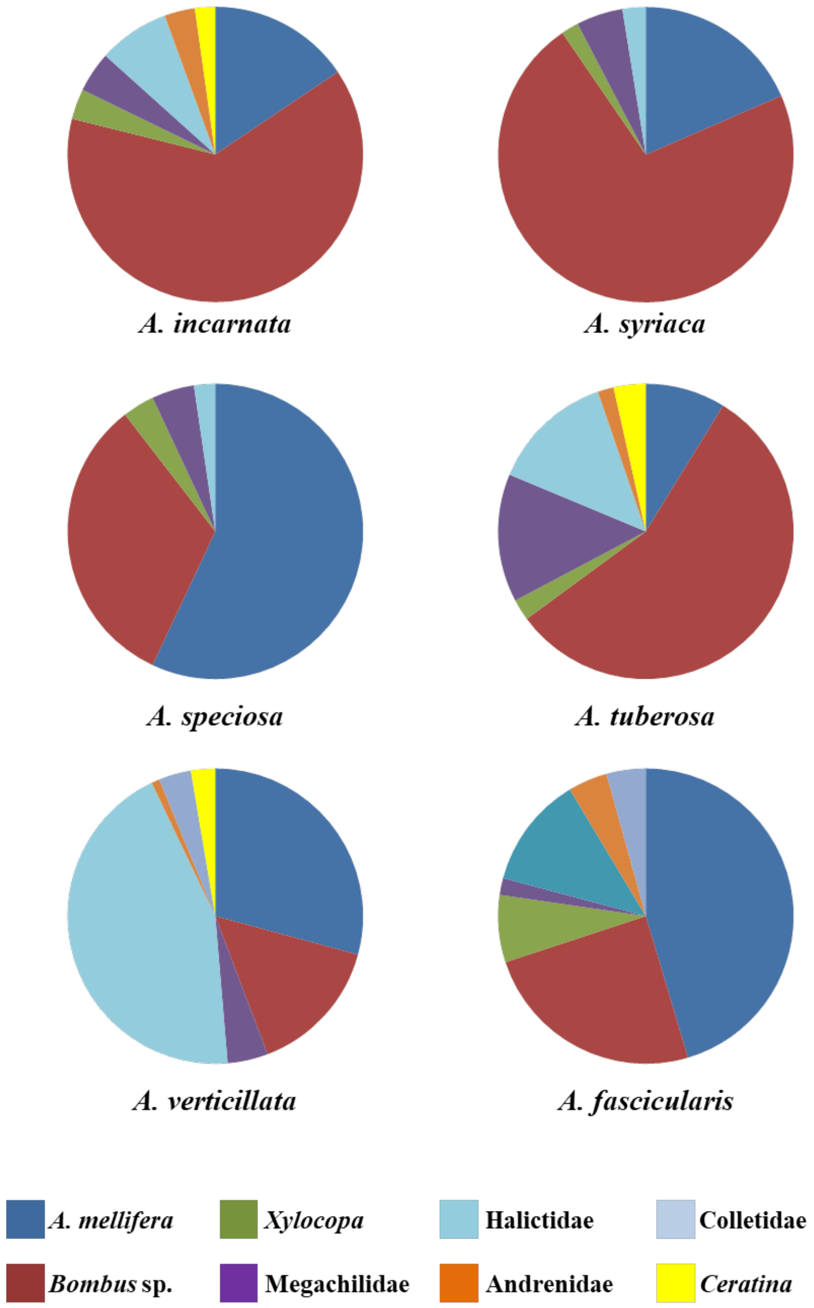

Figure 2.3 Composition of bee samples from the six most bee-attractive milkweed species in the main gardens. Proportions of different taxa (*A. mellifera*, *Bombus* spp., *Xylocopa virginica*, Megachilidae, Halictidae, and combined other groups) differed significantly among milkweed species (Chi-square test for homogeneity; $\chi^2 = 316$, df = 25). See text and Table 4 for genera collected, and genus diversity and richness data

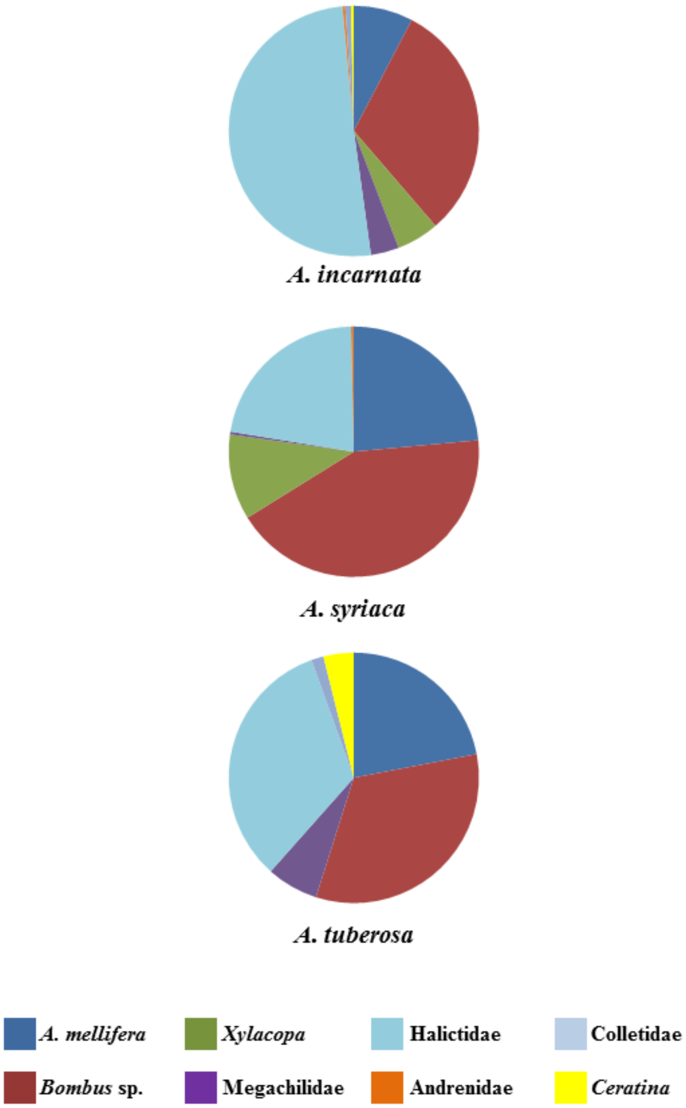

Figure 2.4 Composition of bee samples from *A. incarnata, A. syriaca*, and *A. incarnata* at urban or peri-urban field sites other than the main experimental gardens based on five sites per milkweed species, and 50–55 bees per site. Proportions of taxa differed significantly among milkweed species ($\chi^2 = 104$, df = 10). See text and Table 5 for genera collected, and genus diversity and richness data

Table 2.1 Growth and bloom parameters, and overall attractiveness to bees, of the eight milkweed species evaluated in the replicated garden plots.

Asclepias spp.	Tillers per plot[a]	Mean height (cm)[b]		Bloom		Bloom period 2016 –2017
	2017	2016	2017	2016	2017	
A. fascicularis	103 ± 7 a	82 ± 3 b	105 ± 16 cd	Y	Y	Mid-Jun to mid-Jul
A. incarnata	0.0 ± 0.0 c	91 ± 2 a	109 ± 7 bc	Y	Y	Mid-Jun to mid- Jul
A. latifolia	0.5 ± 0.2 c	43 ± 2 d	60 ± 5.5 e	N	Y	late Jun to early Jul
A. speciosa	6 ± 2 c	79 ± 4 b	138 ± 6. ab	N	Y	May to Jun
A. syriaca	1.0 ± 0.2 c	89 ± 2 a	169 ± 10 a	N	Y	Jun to Aug
A. tuberosa	0.2 ± 0.1c	51 ± 2 c	73 ± 8 de	Y	Y	Late Jun to early Jul
A. verticillata	15 ± 5 b	53 ± 2 c	77±20 de	Y	Y	Mid-Jun to mid-Jul
A. viridis	0 ± 0 c	33 ± 2 e	47 ± 7 e	Y	Y	May to Jul

Data are means ± SE per plot; within columns, means not followed by the same letter are significantly different (ANOVA, Fisher's LSD, $P < 0.05$)

[a] Tillers per plot: $F_{7,28} = 138.72$, $P < 0.001$

[b] Mean height, 2016: $F_{7,28} = 101$, $P < 0.001$; 2017: $F_{7,28} = 14.2$, $P < 0.001$

Table 2.2 Abundance of *Danaus plexippus* eggs and larvae and other specialist herbivores found in biweekly inspections of milkweed plots in five replicated gardens during the 2016 and 2017 growing seasons.

	Monarch larvae + eggs[a]		Aphid rating[b]	
Asclepias spp.	2016	2017	2016	2017
A. fascicularis	3.0 ± 0.7 b	6.6 ± 1.1 ab	1.0 ± 0.0 d	0.3 ± 0.1 c
A. incarnata	15.2 ± 3.0 a	7.8 ± 0.8 ab	3.7 ± 0.4 a	3.4 ± 0.4 a
A. latifolia	1.0 ± 0.4 c	1.4 ± .07 cd	3.0 ± 0.5 ab	3.5 ± 0.4 a
A. speciosa	11.2 ±1.7 a	16.8 ± 6.3 a	1.6 ± 0.4 cd	1.2 ± 0.3 bc
A. syriaca	8.0 ± 0.8 a	12.6 ± 3.4 a	1.6 ± 0.4 cd	1.4 ± 0.6 bc
A. tuberosa	2.0 ± 1.3 bc	5.4 ± 1.7 b	2.0 ± 0.3 bc	0.6 ± 0.2 c
A. verticillata	1.2 ± 0.6 c	0.0 ± 0.0 d	3.0 ± 0.3 a	1.9 ± 0.6 b
A. viridis	1.0 ± 0.3 c	1.4 ± 0.5 c	2.6 ± 0.2 ab	2.0 ± 0.5 b

	O. fasciatus[c]		*Tetraopes* spp.[d]	
	2016	2017	2016	2017
A. fascicularis	14 ± 2 a	22 ± 3 a	0.0 ± 0.0 c	0.2 ± 0.2 b
A. incarnata	4 ± 1 bc	5 ± 1 b	0.6 ± 0.2 b	0.2 ± 0.2 b
A. latifolia	0 ± 0 d	3 ± 2 b	2.2 ± 0.7 a	1.6 ± 0.7 a
A. speciosa	2 ± 0.3 cd	4 ± 1.0 b	0.0 ± 0.0 c	0.4 ± 0.4 b
A. syriaca	11 ± 2 a	43 ± 15 a	0.0 ± 0.0 c	0.6 ± 0.2 ab
A. tuberosa	6 ± 1 b	25 ± 6 a	0.0 ± 0.0 c	0.0 ± 0.0 b
A. verticillata	4 ± 1 bc	9 ± 7 b	1.4 ± 0.2 a	1.6 ± 0.6 a
A. viridis	0 ± 0 d	1 ± 0.5 b	0.0 ± 0.0 c	0.0 ± 0.0 b

Data are mean (± SE) totals per plot

Within columns, means not followed by the same letter are significantly different (Fishers LSD, $P < 0.05$)

[a] Monarch larvae and eggs; 2016: $F_{7,28} = 14.5$, $P < 0.001$; 2017: $F_{7,28} = 14.5$, $P < 0.001$

[b] Aphid rating; 2016: $F_{7,28} = 7.15$, $P < 0.001$; 2017: $F_{7,28} = 8.01$, $P < 0.001$

[c] *O. fasciatus*; 2016: $F_{7,28} = 20.2$, $P < 0.001$; 2017: $F_{7,28} = 8.12$, $P < 0.001$

[d] *Tetraopes* spp.; 2016: $F_{7,28} = 13.6$, $P < 0.001$; 2017: $F_{7,28} = 3.62$, $P = 0.006$

Table 2.3 Performance (weight and instar attained; percentage survival) of cohorts of first or early second instar *D. plexippus* confined on living plants of eight milkweed species in two field trials in the garden plots, and one greenhouse trial.

Field Trial 1 (9-d duration)[a]

Asclepias spp.	Final wt (mg)	Instar attained	% Survival
A. fascicularis	300 ± 122 c	3.6 ± 0.3 c	45
A. incarnata	650 ± 90 ab	4.5 ± 0.2 ab	55
A. latifolia	706 ± 68 ab	4.4 ± 0.1 ab	60
A. speciosa	868 ± 231 a	4.5 ± 0.2 ab	55
A. syriaca	450 ± 39 bc	4.1 ± 0.1 b	50
A. tuberosa	946 ± 196 a	4.7 ± 0.2 ab	65
A. verticillata	1032 ± 140 a	4.7 ± 0.1 a	40
A. viridis	683 ± 218 ab	4.3 ± 0.3 ab	45

Field Trial 2 (7-d duration)[b]

Asclepias spp.	Final wt (mg)	Instar attained	% Survival
A. fascicularis	316 ± 91	3.6 ± 0.2	74
A. incarnata	311 ± 81	3.7 ± 0.2	81
A. latifolia	304 ± 123	3.8 ± 0.3	74
A. speciosa	431 ± 129	4.0 ± 0.2	81
A. syriaca	377 ± 107	4.1 ± 0.3	78
A. tuberosa	359 ± 106	3.8 ± 0.3	59
A. verticillata	320 ± 103	3.6 ± 0.3	74
A. viridis	169 ± 51	3.2 ± 0.1	56

Greenhouse (5-d duration)[c]

Asclepias spp.	Final wt (mg)	Instar attained	% Survival
A. fascicularis	344 ± 59	3.7 ± 0.3	100
A. incarnata	414 ± 38	3.7 ± 0.2	100
A. latifolia	405 ± 55	3.7 ± 0.2	90
A. speciosa	408 ± 47	3.8 ± 0.2	100
A. syriaca	392 ± 63	3.8 ± 0.2	100
A. tuberosa	437 ± 63	3.8 ± 0.3	80
A. verticillata	427 ± 37	4.0 ± 0.0	100

Data are means ± SE

[a] Field Trial 1: $F_{7,25} =$ 3.61, 3.64 for final wt and instar, respectively; $P < 0.01$, $P < 0.01$; within columns, means not followed by the same letter are significantly different (Fishers LSD, $P < 0.05$)

[b] Field Trial 2: $F_{7,62} = 0.52$, 1.19 for final weight and instar, respectively; $P <$ 0.0.8, $P < 0.3$

[c] Greenhouse Trial: $F_{6,51} = 0.57$, 0.46 for final weight and instar, respectively; $P < 0.7$, $P <0.8$; within columns, means not followed by the same letter are significantly different (Fishers LSD, $P < 0.05$)

Table 2.4 Composition of bee assemblages visiting the six most bee-attractive milkweed species in the replicated gardens.

	Milkweed (*Asclepias*) species					
	A. fascicularis	*A. incarnata*	*A. speciosa*	*A. syriaca*	*A. tuberosa*	*A. verticillata*
Andrenidae						
Andrena sp.	7	3	0	0	3	1
Apidae						
Apis mellifera	74	14	49	29	15	33
Bombus bimaculatus	0	0	0	2	0	0
B. griseocollis	38	53	27	110	94	15
B. impatiens	2	4	1	3	2	2
Ceratina sp.	0	2	0	0	6	3
Mellisodes sp.	0	0	0	0	0	1
Xylocopa virginica	12	3	3	3	4	0
Colletidae						
Hylaeus sp.	7	0	0	0	0	4
Halictidae						
Agapostemon sp.	0	1	0	0	2	0
Augochlora sp.	1	1	0	0	2	5
Augochloropsis sp.	7	2	0	0	2	7
Halictus sp.	0	0	0	2	8	1
Lasioglossum sp.	12	3	2	2	9	37
Megachilidae						
Megachile sp.	1	4	2	6	24	4
Osmia sp.	0	0	0	1	0	0
Heriades sp.	2	0	2	1	0	1
Replicates sampled	5	3	3	5	5	4
Total bees sampled	163	90	86	159	171	114
Genus richness	11	10	7	10	12	13
Genus diversity[a] (SE)	0.68* (0.05)	0.56 (0.09)	0.50 (0.09)	0.45 (0.04)	0.65* (0.03)	0.69* (0.07)

[a] ANOVA for genus diversity: $F_{5,15} = 2.93$; $P < 0.05$

*mean is significantly higher than *A. syriaca* (Dunnett's test, $P = 0.05$)

Table 2.5 Composition of bee samples collected on *A. incarnata, A. syriaca*, and *A. tuberosa* at urban or peri-urban field sites other than the main experimental gardens.

	Asclepias spp.		
	A. incarnata	*A. syriaca*	*A. tuberosa*
Andrenidae			
Andrena sp.	1	1	0
Apidae			
Apis mellifera	21	64	56
Bombus bimaculatus	3	1	5
B. griseocollis	79	111	64
B. impatiens	3	4	15
Ceratina sp.	1	0	10
Mellisodes sp.	0	0	0
Xylocopa virginica	15	30	0
Colletidae			
Hylaeus sp.	2	0	4
Halictidae			
Agapostemon sp.	2	0	3
Augochlora sp.	23	0	14
Augochloropsis sp.	0	1	0
Halictus sp.	2	0	11
Lasioglossum sp.	112	59	56
Megachilidae			
Coelioxys sp.	1	0	2
Heriades sp.	3	0	2
Megachile sp.	6	1	13
Osmia sp.	0	0	0
Sites sampled	5	5	5
Total bees sampled[a]	274	272	255
Genus richness	15	9	13
Genus diversity[b]	0.48 ± 0.10	0.42 ± 0.08	0.72 ± 0.02*

[a] Based on samples of 50–55 bees per site during peak bloom

[b] ANOVA for genus diversity: $F_{2,12} = 4.36$; $P < 0.05$; *denotes mean is significantly higher than *A. syriaca* and *A. incarnata* (LSD, $P = 0.05$)

CHAPTER 3

Configuration and location of small urban gardens affect colonization by monarch butterflies

Introduction

Reconciliation ecology, "the science of inventing, establishing, and maintaining new habitats to conserve species diversity in places where people live, work, and play" (Rosenzweig 2003a) aims to modify human-dominated landscapes to support native biota without compromising societal utilization (Rosenzweig 2003ab; Francis and Lorimer 2011). As natural habitats increasingly are cleared, fragmented and degraded by anthropogenic activities, properly designed urban green spaces, including pollinator gardens, can be refuges for native biodiversity, particularly of invertebrates, birds, and other animals able to adapt to human proximity (Goddard et al. 2010; Baldock et al. 2015; Hall 2016; Aronson et al. 2017). Reconciliation ecology also provides opportunities for urban citizens to connect with nature, helping to foster a wider interest in conservation issues (Goddard et al. 2010; Lepczyk et al. 2017). Among insects of conservation concern, none exceeds the power of the monarch butterfly (*Danaus plexippus* L.) to inspire public engagement in reconciliation ecology (Gustafsson et al. 2015).

Instantly recognizable by gardeners and nature lovers, the iconic monarch is renowned for its annual migration in which butterflies from discrete overwintering areas in the highlands of central Mexico recolonize breeding grounds across the United States and southern Canada east of the Rocky Mountains over several generations, followed by a single autumn migration back to Mexico (Reppert and de Roode 2018). The eastern migratory monarch population has declined >80% in the past 25 years (Brower et al. 2011; Vidal and Rendón-Salinas 2014), fueling concern that it may face extirpation unless habitat conservation and restoration efforts are enacted on a continental scale. The monarch population in western North America is also in sharp decline (Schultz et al. 2017). The US Fish and Wildlife Service (USFWS) is currently assessing the monarch's status in response to a petition to list the species under the Endangered Species Act, while working with a broad range of partners as part of an international initiative to conserve the butterfly across its range.

Given that monarch larvae feed exclusively on milkweed (family Apocynaceae, subfamily Asclepiadoideae), and that adults migrate to locate host plants across diverse landscapes, two primary concerns facing monarch populations are shortages of milkweed, and floral nectar to fuel migration (Pleasants and Oberhauser 2013; Oberhauser et al. 2017; Malcolm 2018; Saunders et al. 2019). Conserving and restoring monarch habitat, especially planting of milkweeds and nectar resources on public and private lands, has emerged as the central conservation strategy to meet monarch population goals set by the USFWS and adopted by Mexico, Canada, and the United States. Most research on monarch habitat restoration to date has focused on “non-use” land, e.g., publicly owned grasslands, utility road right-of-ways, Conservation Reserve Program land, edges of fields and pastures, and other marginal habitat (e.g., Kasten et al. 2016; Oberhauser et al. 2017; Pitman et al. 2018). However, restoring enough milkweed to ensure a stable monarch population will require an “all hands on deck” strategy involving participation from all land use sectors including urban and suburban areas (Thogmartin et al. 2017; Johnston et al. 2019). In cities and towns, initiatives such as the Million Pollinator

Garden Challenge, the Monarch Waystation Program, National Wildlife Federation's Butterfly Heroes program, and Mayors' Monarch Pledge are underway, with myriad gardens being planted in backyards, schoolyards, parks, and other public and private places. As of 2019, >25,000 Monarch Waystation habitats (managed gardens containing milkweeds and nectar plants) had been registered with MonarchWatch and the National Pollinator Garden Network had surpassed its goal of registering >1,000,000 pollinator gardens, many likely containing milkweed.

Guidelines for setting up a certified Monarch Waystation recommend that such gardens should have "at least 10 milkweed plants, made up of two or more species," "should contain several annual, biennial, or perennial plants that provide nectar for butterflies," and that "the plants should be relatively close together" because "all monarch life stages need shelter from predators and the elements." Monarchs find and colonize milkweed in urban gardens (Cutting and Tallamy 2015; Baker and Potter 2018; Geest et al. 2019), but little is known about how to configure such gardens to maximize their conservation value.

Ecological theory (e.g., Root 1973; Andow 1991) suggests ways to increase monarch use of milkweed gardens. Susceptibility of plants to attack by insect herbivores may be strongly influenced by the structural and taxonomic complexity of surrounding vegetation (Tahvanainen and Root 1972; Root 1973; Rausher 1981). Dietary specialists, in particular, tend to have difficulty locating host plants growing amongst non-host vegetation, and are less likely to remain on hosts grown in polyculture (Root 1973; Finch and Collier 2000). Mechanisms proposed for such "associational resistance" (Tahvanainen and Root 1972) include visual or olfactory masking, repellent odors, physical obstruction or shading, or inappropriate landings on non-hosts triggering herbivores' premature dispersal (Tahvanainen and Root 1972; Root 1973; Risch 1981; Finch and Collier 2000). Neighboring plants may also provide harborage and food resources for natural enemies (Root 1973; Risch 1981). The aim in polyculture agriculture is to discourage host-finding and colonization by specialist herbivores. The goal for monarch conservation gardens is just the opposite.

We hypothesized that the spatial configuration of host and non-host plants within small gardens, particularly the milkweeds' visual apparency and butterflies' access to them, as well as location of gardens relative to surrounding hardscape, would strongly affect their colonization and use by monarchs. Here, we tested those hypotheses by monitoring (1) monarch use of 22 preexisting citizen-planted Monarch Waystations in relation to those gardens' botanical composition, configuration, and surrounding hardscape, (2) colonization of experimental gardens containing an identical mix of milkweeds, nectar sources, and non-host grasses, but planted in different spatial layouts, and (3) oviposition on isolated milkweeds and milkweeds that were visually obstructed by non-host vegetation.

Materials and Methods

Monarch use of preexisting Waystations

Twenty-two preexisting registered Monarch Waystation gardens were identified via the Monarch Waystation Registry or through the Wild Ones Lexington, Kentucky Chapter, and monitored with permission from landowners or other authorized persons. The Waystations were in residential, commercial, and institutional landscapes, road medians, parks, and nature preserves encompassing a range of anthropogenic settings in and near the cities of Lexington, Richmond, and Berea, in central Kentucky. All of the gardens were mulched, and contained at least three *Asclepias* species, swamp (*A. incarnata*), common (*A. syriaca*), and butterfly (*A. tuberosa*) milkweeds, as well as a variety of annual and perennial flowering plants. Each Waystation was visited twice per month from 5 July to 20 September 2016. Each time, we inspected all milkweeds for monarch eggs and larvae, which were counted and left in place. Monarch eggs and larvae were observed in 20 of the 22 Monarch Waystations.

Monarch Waystations

The Waystations were further characterized by features of the gardens and their surrounding landscape. Garden configuration was classified into two types: “structured” or “non-structured.” In structured gardens ($N = 9$), the milkweeds had been planted in a relatively uniform array, set off by mulch, and separated from neighboring plants by 0.5 m or more. Non-structured gardens ($N = 13$) were also mulched, but had the milkweeds haphazardly intermixed with nectar and non-host plants in no particular arrangement, their foliage often touching or partially shaded by nearby plants. Other garden variables included total area, number of ramets of each milkweed species (counted during bloom when the plants were done producing new ramets for the year), and number of nectar plants.

We used satellite images and the Measure Tool feature of Google Earth Pro geospatial software (Microsoft, Palo Alto CA) to quantify the area of buildings and other hardscape within a 100 m radius centered each garden, the ratio of impervious to pervious surfaces, and distance of the garden to nearby structures. Linear transects were drawn from the garden through corners of all buildings to the edge of the circle. We summed the angles defined by those transects, divided by 360°, and subtracted from 1 to calculate a "360° accessibility index"; i.e., the proportion of access not blocked by buildings if an incoming butterfly approached the garden from 100 m away. Because monarchs fly predominantly northward during their spring migration and south toward their overwintering grounds during fall migration, we hypothesized that unimpeded lines of sight from those directions to resources may be important. Therefore, we determined straight line north/south access by scoring whether or not flight of a butterfly approaching the garden from due north or due south would be blocked by structures.

Monarch use of experimental gardens of differing configurations

Fifteen gardens (5.5 × 5.5 m) were established in spring 2017 in open, non-shaded grassland at the University of Kentucky Spindletop Research Farm in north Lexington, Kentucky. To establish the gardens, plots were sprayed with glyphosate (Roundup ProMax, Monsanto, St. Louis, MO) in April to kill existing vegetation, tilled, and covered with weed barrier cloth. Each garden contained the same mix of swamp milkweed, nectar plants, and ornamental grasses in one of three different spatial configurations, representing treatments: (1) milkweeds evenly spaced in a 1 m wide corridor around the perimeter with nectar plants and grasses in the interior (Figure 3.1A); (2) nectar plants and grasses in a 1 m corridor around the perimeter with milkweed in the interior (Figure 3.1B); or (3) random arrangement of all plants without formal garden structure (Figure 3.1C), hereafter referred to as gardens with "perimeter milkweeds," "interior milkweeds," and "mixed," respectively. Gardens were placed on 300 m transects

(100 m spacing between treatments) oriented on an east-west axis within each replicate to minimize bias in their likelihood of being encountered during flight of north or south bound monarch butterflies. Each of the five replicates was separated by at least 300 m.

We used swamp milkweed, *A. incarnata*, because it grows to a consistent height of about 1 m and does not spread via rhizomes (Baker and Potter 2018). Two-year old potted plants (30 cm tall) were transplanted (12 per garden) in early May 2017. To increase the structural and taxonomic complexity of the vegetation surrounding the milkweeds, each garden also contained flowering annuals differing in height and form, including Mexican sunflower, *Tithonia rotundifolia* (12 per garden) and common zinnia, *Zinnia elegans* “Canary Bird” (12 per garden), which are attractive nectar sources for adult monarchs, and ornamental feather reed grass, *Calamagrostis* × *acutiflora* (four per garden). Mexican sunflower grows to 1.2–1.5 m height and 0.6–0.9 m spread; *Z. elegans* to 0.6–0.9 m height and 0.2–0.3 m spread, and *Calamagrostis* reaches 0.9–1.5 m height and 0.45–0.76 m spread[8]. Nectar plants were greenhouse-grown from seeds (Applewood Seed, Arvada, CO), whereas the ornamental grasses were purchased in 11.5 liter pots (Baeten's Nursery, Union, KY).

For gardens with perimeter milkweeds, the 12 *A. incarnata* were planted with even spacing in the 1 m border, 1.5 m apart, and the *Tithonia, Zinnia*, and *Calamagrostis* were evenly spaced within the inner block with one grass transplanted at each of the four cardinal directions (Figure 3.1A). For gardens with interior milkweeds (Figure 3.1B), the 12 *A. incarnata* were spaced 1.1 m apart in the inner block, with the *Tithonia* and nectar plants alternated evenly around the perimeter in the 1 m border, and for mixed gardens (Figure 3.1C), all plants were assigned to random distribution over the whole plot. Each garden received a 5 cm deep layer of dark-brown mixed hardwood mulch over the entire plot and surrounding all plants. The gardens were watered to maintain plant vigor for a month after planting, but received only natural rainfall for the duration of the study. They were hand-weeded, and re-mulched at the start of the second (2018) growing season, at which time a few of the less-vigorous milkweeds were replaced with similar-sized healthy 2-year-old plants. The grass (mostly tall fescue, *Festuca arundinacea*) surrounding each garden was mowed weekly to 10 cm height.

Assessing monarch colonization and use of gardens

Gardens were inspected for all monarch life stages during the 1st and 3rd week of each month from June to September 2017, and during the 2nd and 4th week of each month beginning 9 April until 23 July 2018, when a severe storm uprooted the taller, mostly *Tithonia* nectar plants, reducing integrity of the treatments. At each visit we carefully inspected above-ground portions of each milkweed by examining the stems, and the top and bottom of each leaf for monarch eggs, larvae, and pupae which were counted and left in place.

Natural enemy abundance in gardens

Two methods were used to assess if garden design influenced abundance of generalist invertebrate predators in the gardens. First, all above-ground portions of the 12 milkweeds in each garden were inspected every two weeks from June to September 2017, and April to July 2018 on alternate weeks from when monarch life stages were counted.

We recorded numbers of adults and immatures belonging to predominantly predatory taxa on each plant, spot-identifying to order and family and leaving them in place.

Abundance of ground-dwelling predators that monarch larvae might encounter while moving between plants or to pupation sites was assessed using pitfall traps deployed for 48 h from July 19-21 and July 26-28, 2018, during peak monarch activity. Traps consisted of 0.47-liter plastic cups, with 2 cm of ethylene glycol as a killing agent, set into the ground with the brim 2 cm below the surface. There were four traps per garden spaced at least 2 m apart, but within 1 m of the milkweed. Trapped invertebrates were stored in 70% ethanol, and sorted and identified to order and family.

Effect of surrounding vegetation on susceptibility of milkweeds to oviposition

A supplemental experiment investigated how presence or absence of surrounding non-host vegetation affects a milkweed plant's susceptibility to monarch oviposition. The trial ran from 6 to 21 August 2018 in an open grassy area of the University of Kentucky State Botanical Garden and Arboretum (38°00′57.5″N 84°30′15.7″W), Lexington, KY. Six pairs (replicates) of *A. incarnata* (about 90 cm tall) in 4 liter pots were sunk into the soil so that the pot rims were even with the ground surface. Plants within replicates were spaced 9 m apart along an east-west transect, with replicates separated by at least 11 m. One randomly-chosen milkweed of each pair was surrounded by three clumps of ornamental grasses, *Panicum virgatum* “Shenandoah,” in 11 liter pots that were placed in a triangular array at 0.6 m distance. The uppermost foliage of the grasses and milkweeds was at similar height, with their foliage separated by about 0.5 m, but the grasses close enough that they might form a visual screen to monarchs flying over the landscape in search of milkweed for oviposition. The milkweeds were inspected daily for monarch eggs, and at each visit, such eggs were removed.

Statistical analysis

Data relating the characteristics of the preexisting Monarch Waystations and total number of monarch eggs and larvae found in those gardens were analyzed by multivariate analysis of variance (ANOVA) using the Statistical Analysis System general linear models procedure (SAS, Version 9.4; SAS Institute, Cary, NY, USA) to test for associations between monarch abundance and garden characteristics including area, milkweed density, nectar plant density, and whether or not the garden configuration was structured or non-structured, as well as surrounding landscape features within a 100 m radius of the garden including % hardscape, number, and total area of buildings, distance to nearest building, 360° accessibility index, and north/south accessibility. We used stepwise model selection to omit independent variables not producing a significant F-statistic and calculate adjusted r^2 values for the full and reduced models.

Counts of monarch life stages on the milkweeds were summed across sample dates, within year, and those totals were compared between garden layouts by two-way (ANOVA) for a randomized complete block design using Statistix 10 (Analytical Software, Boca Raton, FL). Direct counts of predatory invertebrates on the milkweeds, and numbers captured in the pitfall traps, were similarly analyzed for each data set, as were numbers of monarch eggs deposited on milkweeds that were or were not surrounded by ornamental grasses. Log or square root transformations were used if needed to meet normality and homogeneity of variance assumptions. Data are reported as original means ± standard error (SE).

Results

Monarch use of preexisting Waystations

Multivariate analysis of variance for predictors of monarch egg and larval abundance in the 22 citizen-planted Monarch Waystations explained 63 and 71% of the variation with complete and reduced models, respectively (Table 3.1). Stepwise model selection identified three factors: garden configuration, north/south accessibility, and proximity to nearest building as significant sources of variation. Total numbers of monarch eggs and larvae observed in twice-monthly visits to each garden were about five-fold higher in structured gardens with spacing between milkweeds and non-host plants than in non-structured gardens where those plants were closely intermixed (Figure 3.2A), and similarly higher in gardens with unobstructed north-south access compared to ones where such access was obstructed by buildings (Figure 3.2B). There was also a positive relationship between monarch abundance and proximity to the nearest structure. Other features of the gardens themselves (area, density of milkweeds, or nectar plants) or of the surrounding landscape within a 100 m radius did not explain a significant amount of variance in use by monarchs (Table 3.1). The gardens varied with respect to percentage of surrounding area occupied by hardscape (5–78%) and degrees of 360° access impeded by buildings or other structures (0–360°).

All 22 gardens contained *A. incarnata, A. syriaca*, and *A. tuberosa* which were nearly equally represented (Figure 3.2C). Two gardens also contained one or two plants of *A. verticillata* (whorled milkweed), but no other milkweed species were represented. Total milkweed ramets per garden averaged 54 ± 8.7 (range 10–198). Total numbers of eggs and larvae found in the six, twice-monthly inspections averaged 13.3 ± 3.9 per garden, with high variability (range 0–61) between garden sites. Across all gardens, we found a total of 137, 134, and 11 monarch eggs and larvae on 380, 437, and 312 ramets of *A. incarnata, A. syriaca*, and *A. tuberosa*, respectively, with proportionately more on *A. incarnata* and *A. syriaca* than on *A. tuberosa* ($\chi^2 = 109.0$, $P < 0.001$; Figure 3.2D). Monarch abundance (total for all garden counts) built up over the growing season, peaking in September.

Monarch use of experimental gardens of differing configurations

In both 2017 and 2018 monarch eggs and larvae were 2.5–4 times more abundant in gardens in which the milkweeds were planted around the perimeter, surrounding the nectar plants and grasses, than when the layout was reversed, with milkweeds in the garden interior, or when the milkweeds were randomly intermixed with the other plants (Figure 3.3).

All three garden configurations harbored similar communities of predatory arthropods. Lady beetle adults and larvae (Coccinellidae), lacewings (Chrysopidae), and spiders (Araneae) were the most abundant predators observed on the milkweed plants (Figures 3.4A,B) with smaller numbers of ants, predatory Hemiptera (Pentatomidae, Reduviidae, and Nabidae) and others. Direct counts on the milkweeds did not differ among garden types for any predator group (Figures 3.4A,B; $F_{(2,8)} \leq 1.7$ for all individual taxa; all $P \geq 0.24$). Ground-dwelling predators captured in pitfall traps included ants, spiders, ground beetles (Carabidae), rove beetles (Staphylinidae), harvestmen (Opiliones), and other groups (Figure 3.4C). Garden design had no effect on activity-density of any of those groups ($F_{(2,8)} \leq 1.5$ for all individual taxa; all $P \geq 0.27$).

Effect of surrounding vegetation on susceptibility of milkweeds to oviposition

Female monarchs foraging in an open-field setting laid significantly more eggs on single milkweed plants that were accessible from top to bottom, without visual obstruction, compared to single plants surrounded by, but not touching, ornamental grasses of equal height (Figure 3.5). Milkweeds screened by the grasses received almost no eggs over the 2-week trial.

Discussion

Numerous programs encourage individual landowners, citizen scientists, and organizations in residential areas to establish gardens with milkweed and nectar plants to help offset habitat loss across the monarch's breeding range, and to increase connectivity among habitat patches in other land types. Optimizing the conservation value of such gardens is important because of the substantial effort and resources being directed toward them, and because restoring monarchs to a population goal specified in the North American Monarch Conservation Plan will likely require contributions from all land use sectors (Pleasants 2017; Thogmartin et al. 2017). Indeed, geospatial extrapolations indicate that if all metropolitan areas across the US eastern range were engaged, they could provide nearly a third of the projected milkweed needed to sustain the eastern monarch population (Johnston et al. 2019).

To contribute to monarch conservation, gardens must first attract females to lay eggs. Monarchs find and oviposit on milkweeds in small urban gardens, often with higher egg-loading per plant than in natural habitats (Cutting and Tallamy 2015; Stenoien et al. 2015; Baker and Potter 2018; Geest et al. 2019). The present study indicates that the layout of such gardens strongly influences the extent to which the milkweeds therein are found and used. Results from each of its components; i.e., numbers of eggs and larvae in existing Monarch Waystations, colonization of replicated gardens with different configurations, and oviposition on milkweeds with or without surrounding non-host vegetation, support the hypothesis that at least within small gardens, milkweeds are more susceptible to discovery and oviposition when they are spatially separated from nectar and non-host plants as opposed to being closely intermixed with them.

Host-finding by most butterfly species involves a sequence of behaviors including habitat location, orientation, landing, and plant surface evaluation (Renwick and Chew 1994). Monarch adults are highly vagile and move extensively between habitat patches with milkweeds and nectar plants, but the relative distances over which they use visual or olfactory cues to locate resources are poorly understood (Zalucki et al. 2016). Caged lab-reared monarchs learned to associate the color and shape of artificial flowers with a nectar reward in the laboratory (Cepero et al. 2015), suggesting they also use such visual cues when orienting to hosts in the field. Upon landing, females engage contact chemoreceptors on their antennae and tarsi to assess plant suitability for oviposition, with flavanol glycosides in asclepiad hosts serving as oviposition stimulants (Baur et al. 1998). Monarchs encountering natural stands of milkweed tend to lay more eggs on taller plants than on shorter ones, and more eggs per plant on isolated plants, and on plants at the edge of a patch compared to ones in a patch center (Zalucki and Kitching 1982a,b; Zalucki et al. 2016).

In our study the gardens were standardized by area and botanical composition. All gardens contained the same number of milkweeds, but the interplant distances between milkweeds differed and were systematically greater in the “perimeter milkweed” layout than in the other garden designs. Because monarchs are known to preferentially oviposit on isolated milkweeds, this may have influenced the results. Our purpose, however, was to find ways to optimize monarch use at the whole-garden scale by comparing same-sized gardens planted in different configurations. Consistent with Pitman et al. (2018), who found higher egg densities in small (<16 m^2), low-density (0.1–2 milkweed per m^2) milkweed patches in agricultural areas than in larger, higher-density milkweed patches, our small experimental gardens and surveyed Monarch Waystations were readily colonized and used by monarchs.

Visual and chemical stimuli from host and non-host plants can affect specialist herbivores' ability to find and colonize habitat patches, and their behavior in those patches (Tahvanainen and Root 1972; Root 1973; Risch 1981; Finch and Collier 2000; Bruce et al. 2005). The strength of attractive stimuli for a particular herbivore determines what Root (1973) called “resource concentration” which is affected in turn by density and spatial arrangement of host and non-host plants, and potential interference from non-hosts. (Root 1973).

Resource Concentration Hypothesis predicts that a specialist herbivore approaching a habitat will have greater difficulty locating a host plant when the relative resource concentration is lower. Non-host vegetation may impair specialists' host-finding by physical obstruction, visual camouflage, making it more difficult for the herbivore to identify correct blends of volatiles produced by host plants against a complex background of volatiles from non-hosts, shading, or otherwise causing host plants to become less attractive or suitable (Tahvanainen and Root 1972; Root 1973; Risch 1981; Bruce et al. 2005). Moreover, “inappropriate” landings on non-hosts may cause specialists to emigrate more quickly from mixed-plant habitat patches of low resource concentration (Root 1973; Risch 1981; Finch and Collier 2000). There is evidence that monarchs are more likely to find and oviposit on milkweeds growing in monoculture agricultural fields than on milkweeds embedded in more botanically diverse habitats such as roadsides, nature preserves, and prairies (Pleasants and Oberhauser 2013).

Some other diurnal specialist butterflies (e.g., the pipevine swallowtail *Battus philenor*) that use visual cues, e.g., leaf shape, when approaching host plants for oviposition have more difficulty locating hosts growing amid non-host vegetation than when such vegetation is removed (Rausher 1981). A similar phenomenon, involving both visual camouflage and physical obstruction, may explain the results from this study. Results of our trial comparing oviposition on individual milkweed plants surrounded or not surrounded by non-host grasses also support the visual camouflage/physical obstruction hypothesis.

Resource concentration and accessibility may also help to explain why female monarchs moving amongst natural patches of milkweed tend to lay more eggs on relatively taller, single, isolated, or edge plants (see above). Indeed, Zalucki and Kitching (1982b) predicted that once a female finds a habitat patch, her movements will be determined by local environmental stimuli; e.g., host plant spacing, flowering plants, and edges, as well as her physiological condition. Those movements determine patch use, and how quickly a patch is "lost" by the butterfly wandering out of it.

An alternative hypothesis for why we found fewer monarch eggs and larvae in gardens having the milkweeds closely intermixed with nectar and non-host plants is that predatory invertebrates might be more abundant in such gardens, or might more readily move from non-host plants to prey on monarchs on adjacent milkweeds. However, our pitfall traps and direct inspections of milkweed plants found no evidence that garden design affected abundance of any predator group. We did not measure parasitism, or losses to birds, vespid wasps, or other flying predators, but there is no reason to expect those mortality agents would be any more or less prevalent in gardens having different layouts of the same plants. Indeed, visually-searching predators would seemingly have less difficulty finding monarch larvae on milkweeds not intermixed with other plants which, if affected by garden configuration, would have contributed to per-garden populations opposite of what we found.

Of those landscape features we analyzed, unimpeded north-south access to gardens was the strongest predictor of monarch egg and larval abundance in citizen-planted Monarch Waystations. Although monarchs foraging locally may approach and leave milkweed patches from all directions (Zalucki and Kitching 1982b), unimpeded north/south access to gardens may be particularly important for them to be encountered and used when adults are flying predominantly southward during their fall migration or northward during spring migration. North-south access may also be important because availability of nectar sources, particularly during autumn migration, may be critical to monarchs' migration success (Saunders et al. 2019). Interestingly, neither overall percentage of hardscape within a 100 m radius of the gardens, nor the percentage of total (360°) access blocked by buildings, was a significant determinant of monarch use. Several of the gardens with relatively high numbers of monarchs were located close to the east or west side of buildings, which may account for the positive correlation between those factors in the multivariate analysis. Orientation of a garden in relation to structures, not the proximity *per se*, may affect monarch use. Nevertheless, the two least productive Waystations we surveyed were the only ones located in courtyards where access to them was blocked by structures. Further research on monarch foraging in relation to hardscape and other features of urban landscapes is warranted.

Despite the public's high level of enthusiasm and capacity for monarch-friendly gardening and projections that the urban sector can make important contributions to monarch recovery (Thogmartin et al. 2017; Johnston et al. 2019), the conservation value of such gardens remains uncertain. That urban milkweed gardens have the potential to recruit monarchs, often with higher egg-loading per plant than occurs in natural milkweed stands, is established (Cutting and Tallamy 2015; Stenoien et al. 2015; Baker and Potter 2018; Geest et al. 2019). Such gardens, however, could serve as ecological traps if they expose monarch larvae to increased risk of predation, disease, or pesticides (Majewska et al. 2018; Geest et al. 2019). We did not measure egg or larval survival, but earlier studies found no difference in overall survival (Cutting and Tallamy 2015), or in mortality from parasitic tachinid flies or the protozoan *Ophryocystis electroscirrha* (Geest et al. 2019) between urban gardens versus more natural sites in meadows or conservation reserves, respectively. We have documented high rates of European paper wasp, *Polistes dominula*, predation on monarch larvae in some urban gardens (Baker and Potter unpublished). Given the propensity of this wasp to nest in building eaves, cavities, and other sheltered places associated with human structures (Liebert et al. 2006), it could potentially pose a greater hazard to monarchs in urban settings than in more natural ones.

Regardless of their value in helping to restore the eastern migratory monarch population, Monarch Waystations and similar gardens provide opportunities to engage large numbers of people in reconciliation ecology. While the magnitude of the current extinction crisis is widely recognized by scientists (IPBES, 2019), we are witnessing an “extinction of experience” (Pyle 1993; Miller 2005; Goddard et al. 2010) whereby the US general public, 80% of which now lives in metropolitan areas, is increasingly estranged from the natural world. Gardening for monarchs, whether by individual landowners, school children, or organizations, can help foster personal engagement with nature, providing social and educational connections that enrich urban residents' quality of life, and engendering public support for protecting native species (Miller 2005; Goddard et al. 2010). Our findings suggest guidelines for designing small gardens that can help make the urban sector's contributions to monarch habitat restoration more rewarding for participants, and of greater value to monarch recovery.

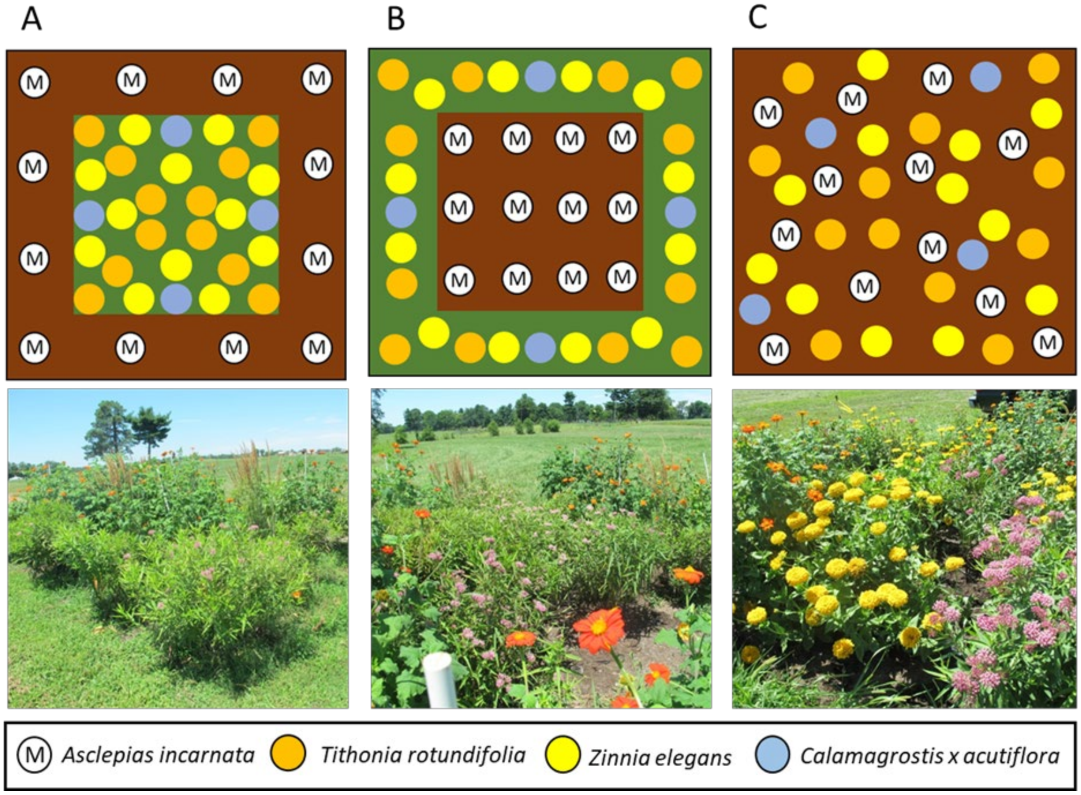

Figure 3.1 Layout of the three garden designs tested. Top row, left to right: (A) milkweed plants on the perimeter of the garden (M), spacing with mulch (brown), nectar/camouflage plants on interior of garden [*Tithonia rotundifolia* (orange), *Zinnia elegans* (yellow), and *Calamagrostis x acutiflora* (blue)]; (B) milkweed on the interior of the garden and placement of the nectar/camouflage plants on exterior of garden; (C) no formal design to simulate a naturalized or mixed garden. Milkweed and nectar/camouflage plants were placed randomly throughout each quadrant in the gardens. Bottom row, left to right: gardens of the aforementioned designs, respectively, as they appeared in 2018.

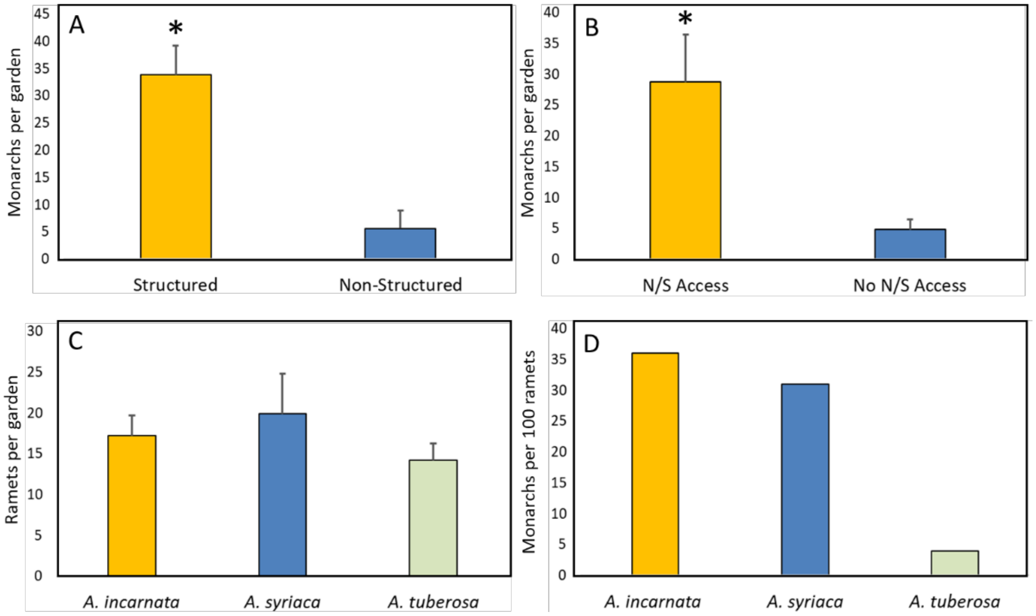

Figure 3.2 Summary data from season-long survey of citizen-planted Monarch Waystations ($N = 22$): (A) Mean total monarchs (eggs and larvae) in structured gardens (milkweeds in uniform array, separated from other plants by ≥0.5m) or non-structured gardens (milkweeds closely intermixed with non-host plants); (B) Mean total monarchs (eggs and larvae) in gardens with or without unimpeded north-south access to 100 m: (C) Mean total ramets per garden of the three predominant milkweed species; (D) Mean total monarch eggs and larvae per 100 ramets of each milkweed species. Asterisk denotes significant difference. See text and Table 1 for statistical comparisons.

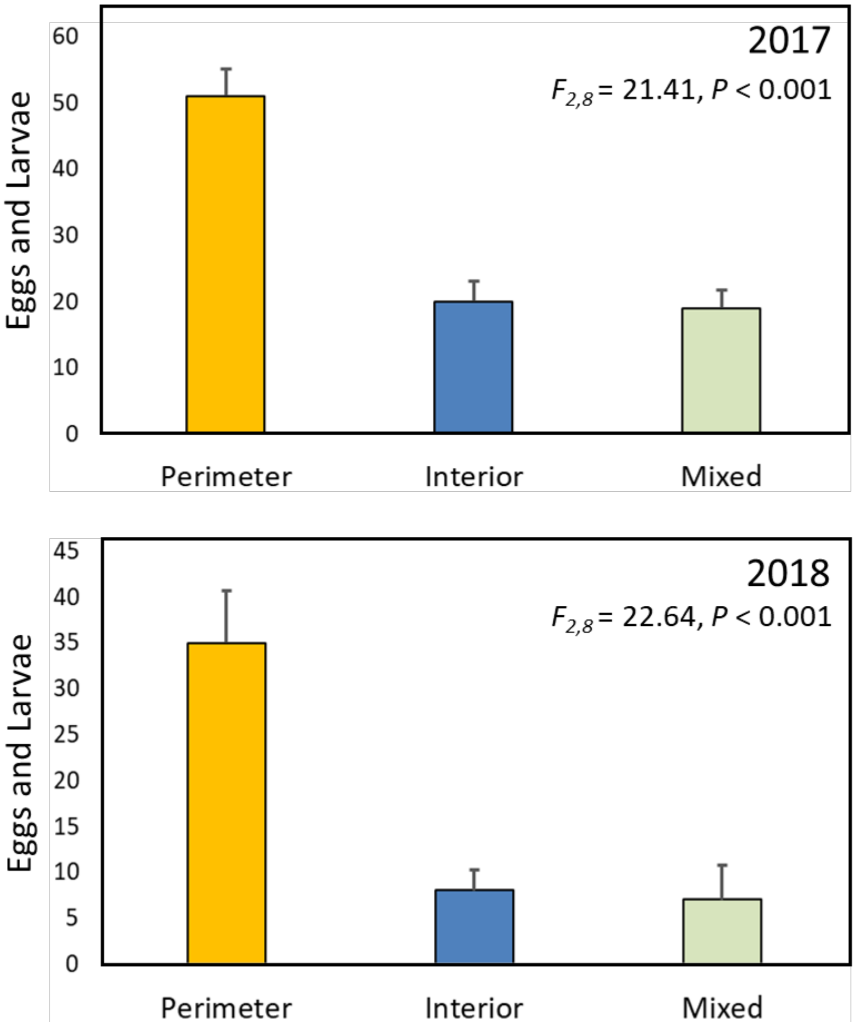

Figure 3.3 Mean (± SE) total monarch eggs and larvae per garden for the three garden designs described in Figure 1.

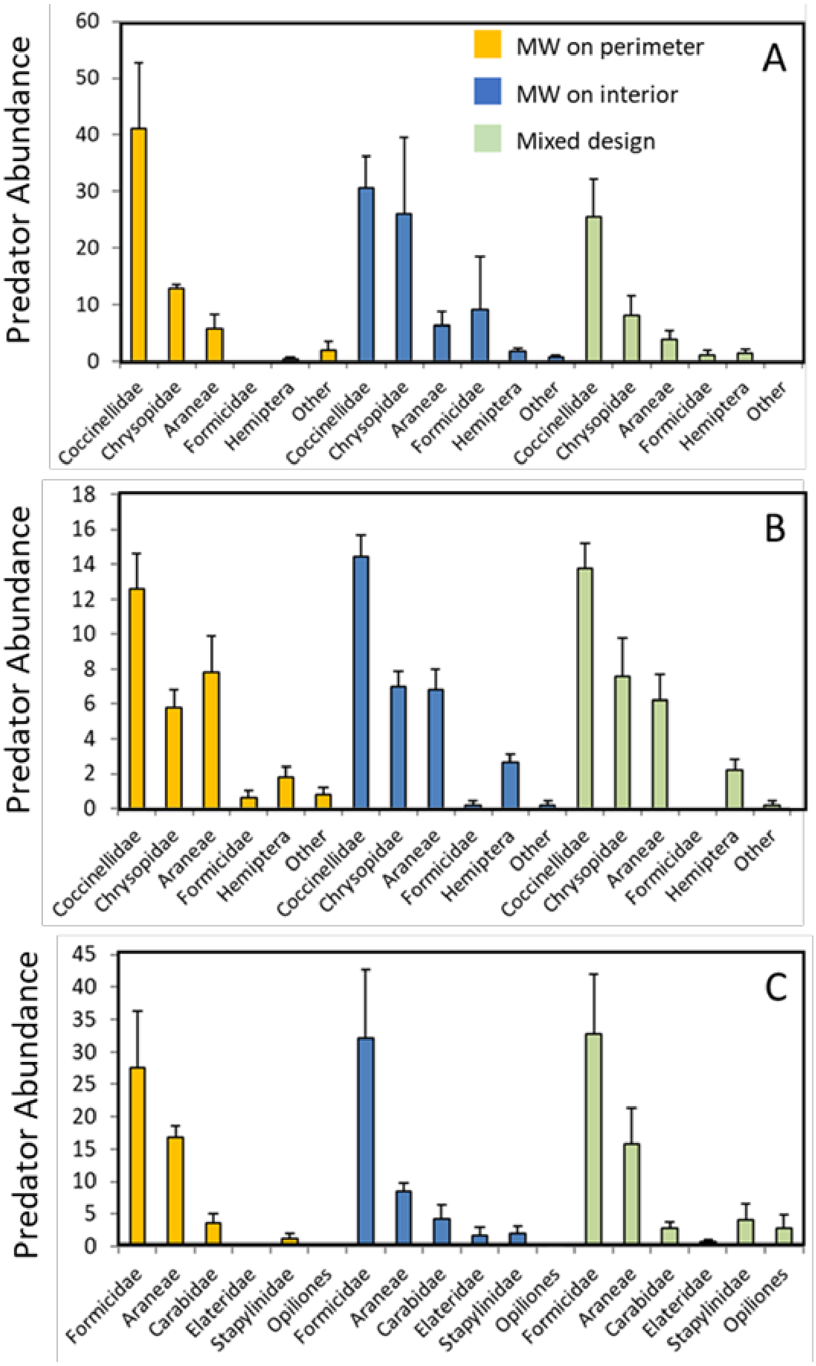

Figure 3.4 Predator abundance by garden design; Milkweed (MW) on perimeter (orange), Milkweed on interior (blue), Milkweeds intermixed (green). (A) Predator groups observed on host plant foliage in gardens (2017); (B) Predator groups observed on host plant foliage in gardens (2018); (C) Predator groups collected in pitfall traps in the gardens (2018). Counts are means (SE) per garden treatment combined. Garden design did not significantly affect counts of any predator group (ANOVA, all $P \geq 0.24$).

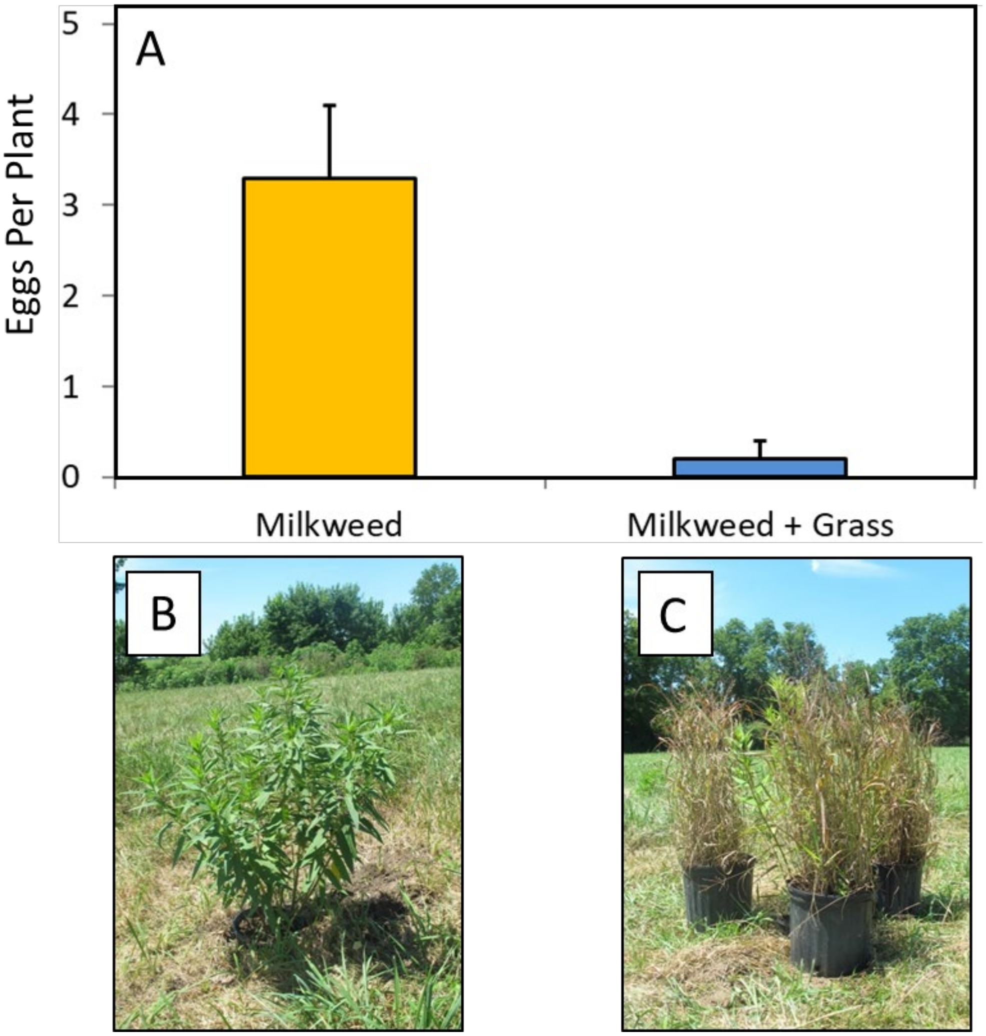

Figure 3.5 (A) Abundance of monarch eggs observed on isolated milkweed plants and milkweeds visually obstructed by ornamental grasses. (B) Isolated potted milkweed set at ground level. (C) Milkweed visually obstructed by ornamental grasses. Means for isolated vs. obstructed plants differ significantly ($F_{1,5} = 17.87$, $P < 0.01$).

Table 3.1 Summary of analysis of variance for the effects of garden characteristics and landscape features on the number of monarch eggs and larvae observed in gardens. Adjusted r^2 full model; 0.63, reduced model; 0.71

Garden Characteristics[1]	**df**	***F***	**Pr>*F* (full)**	**Pr>*F* (reduced)**
Garden Area	1	0.02	0.89	----
Milkweed Ramet Density	1	1.35	0.27	----
Nectar Plant Density	1	0.39	0.55	----
Plant Separation	1	16.49	<0.01	<0.01*
Landscape Features[2]				
Accessibility Index 360°	1	0.35	0.57	----
Line of Sight North/South	1	5.42	0.04	<0.01*
Area Occupied By Structures	1	1.37	0.27	----
% Hardscape	1	1.75	0.21	----
Nearest Structure to Garden	1	5.95	0.33	0.01*
Number of Structures	1	0.39	0.54	----

[1] Garden area (m^2), milkweed ramet density, nectar plant density, plant spacing (use of mulch to achieve plant separation) in garden

[2] All measurements based on 100 m radius buffer zone around center of gardens. Accessibility index (degrees visually obstructed out of 360°), line of sight north/south (visual obstruction north/south), area occupied by structures (% of buffer zone), % hardscape (includes buildings and any impenetrable surfaces), nearest structure to garden, number of structures

Significant variables that were retained from the full model during stepwise model selection indicated by (*)

CHAPTER 4

Native milkweed cultivars provide conservation value for monarch butterflies and bees in urban gardens

Introduction

Monarch butterflies and native bees are declining across North America (Goulson et al. 2015; Koh et al. 2016; Brower et al. 2012; Rendon-Salinas et al. 2016). The public has rallied to help their plight by establishing millions of gardens with flowering plants to provide nectar and pollen, and host plants to support butterfly larvae (MPGC 2020; MonarchWatch 2020). Enthusiasm for pollinator and wildlife gardening has ignited a fervent native plant "movement" (Tallamy 2007). Urban landscape plants, regardless of their provenance, can support biodiversity by providing floral resources for pollinators (e.g., Salisbury et al. 2015; Mach and Potter 2018) as well as seeds, fruits, and insects that serve as food for birds and other desirable urban wildlife (Goddard et al. 2010; Henning and Ghazoul 2012). Nonetheless, because native insects have had millions of years to adapt to the chemical defenses of sympatric native plants, the latter often support higher abundance and diversity of butterfly larvae, and of arthropods needed by insectivorous birds to raise their young (Zuefle et al. 2008; Burghardt et al. 2009; Narango et al. 2017). Thus, there is debate whether use of non-native garden or landscape plants, even non-invasive ones, is anathema to supporting urban biodiversity.

The adoption of native plant landscapes is fueling a major trend in plant marketing – their promotion and use in the landscape, garden, design, and retail trades. Wild-type natives, however, aren't the only options on the table; native plant cultivars, often referred to as 'nativars', are gaining attention, too (Hanson 2017; Curry 2018). Such plants, natural variants of native species that are selected and propagated for desirable attributes such as plant stature, color, disease resistance, or bloom period, open the door to new introductions that provide consumers the best attributes of natives and ornamentals combined.

Native plant cultivars, nevertheless, are not without controversy, and conservation groups want to know "do they serve the same ecological functions and provide the same benefits to bees, butterflies, birds, and other biodiversity as wild-type native plants?" Some environmental organizations (e.g. Marinelli 2016) decry 'nativars', arguing that mass-production, promotion, and use of cultivars instead of wild-type strains could diminish the genetic diversity of urban forests, landscapes, and gardens, reducing plants' capacity to adapt to change, support wildlife, or provide other environmental services. While it's true that some selections of native plants, e.g., *Hydrangea arborescens* 'Annabelle' selected for large clusters of sterile, white flowers, provide no floral resources for pollinators, other native plant cultivars do provide high-quality nectar and pollen and can be equally or more attractive to pollinators as their wild-type ancestors (Salisbury et al. 2015; Nevison 2016; Mach and Potter 2018). Previous research found no evidence that cultivars of native woody plants selected for enhanced fruiting, leaf variegation, disease resistance, or altered growth habit supported fewer insect herbivores than wild-types, or that they would degrade insect-based food webs if more widely grown (Baisden et al. 2018).

The rise of 'nativars' in the marketplace is being driven by interest in using native plants for ecological gardening, and by consumer demand for novel native plants that are both attractive and different from the ordinary. With pollinator and wildlife conservation driving the debate over whether or not 'nativars' have a place in native plant gardening, the answer, based on limited studies so far, is "it depends" (White 2016; Baisden et al. 2018; Ricker 2019). Given the vast potential market for new consumer-attractive cultivars of native plants, growers and garden centers need research-backed information to better answer customers' questions about whether or not such plants are compatible with their gardening goals.

The 2019-2020 census of the monarch overwintering grounds revealed a 53% reduction in monarch populations from the previous year (MonarchWatch 2020). With the ongoing goal of maintaining 6 hectares occupied by overwintering monarch butterflies, the 'all hands on deck' conservation efforts continue (Thogmartin et al. 2017), meaning another productive year for milkweed sales. Each season more milkweed cultivars, the obligate host plant of the monarch, are released for consumers to use in conservation gardens. Plants that are being marketed for use in habitat creation, especially those that are host plants for desirable and declining fauna, need to be evaluated to ensure that they are suitable for use in conservation. In this study we use the monarch and milkweed system to test the hypothesis that nativars are suitable host plants for monarchs and bees in garden settings.

Methods

Study sites

Field. In May 2018, we established six replicated gardens (1.22 x 9.75 m) in public areas of the Arboretum State Botanical Garden of Kentucky, Lexington Kentucky. Each garden contained wild-type milkweeds and three cultivars of each of the two species including *A.*

incarnata wild-type, 'Ice Ballet', 'Soulmate' and 'Cinderella', and *A. tuberosa* wild-type, 'Blonde Bombshell', 'Gay Butterflies', and ‘Hello Yellow’ (Figure 4.1). Milkweeds were purchased from various producers (American Meadows, Shelburne, VT; Centerton Nurseries, Bridgeton, NJ; Prairie Moon, Winona, MN) as bare root 2-year old plants which were started in the greenhouse. To establish the gardens, plots in open grassland were sprayed with glyphosate in April to kill existing vegetation, tilled, and covered with weed barrier cloth. Milkweeds were transplanted into gardens and 5 cm of dark-brown hardwood mulch was added. We subdivided each garden into eight randomized plots (1.22 × 1.22 m), one for each of the eight milkweed types. Four milkweeds (16–30 cm height) were transplanted 0.6 m apart within each plot (six replicates; 24 total plants of each of the eight types). We replaced some of the less-vigorous milkweeds with healthier greenhouse grown transplants in May 2019.

Greenhouse. All larval performance experiments were conducted at the University of Kentucky greenhouses. The temperature was regulated at 27°C and no artificial light was used. All plants were grown in 5.6 liter pots, using a soil and bark mix (SunGro, Quincy, MI). In addition to the *A. incarnata* and *A. tuberosa*, we tested commonly available non-native milkweeds including Balloon Plant (*Gomphocarpus physocarpus*), tropical milkweed (*A. curassavica*) and three of its cultivars ‘Charlotte’s Blush’, ‘Silky Gold’, and ‘Silky Deep Red’.

Monarch colonization of gardens

Milkweeds in each garden were monitored for monarch eggs and larvae twice monthly from June- September 2018 and May-August 2019. At each visit all plants were inspected by turning over all leaves, and also examining stems and flowering portions of the plant.

Larval performance on milkweeds

Performance of monarch larvae on all milkweed types was tested in two greenhouse trials conducted in July 2019. Trial 1 included two year old rootstock (same as garden milkweeds) of *A. incarnata* and *A. tuberosa* and their cultivars. *Asclepias tuberosa* ‘Blonde Bombshell’ was not included in this experiment because of poor regeneration and market unavailability. Trial 2 included *G. physocarpus* and *A. curassavica* and its cultivars. All milkweeds were 30–60 cm tall. Newly-molted second instars were placed on plants (one per plant; 10 replicates each) and caged using white fine mesh bags (25 × 40 cm). Plants were randomized within each replicate once per day. Larvae were allowed to feed for 7d and then evaluated for amount of weight gained and larval instar achieved.

Defensive characteristics of milkweeds

Trichome densities and latex exudation were compared among milkweeds by the methods of Agrawal and Fishbein (2006). In June 2019, four leaves from each replicate (24 total per plant type) were collected, leaf discs (28 mm^2) were taken from the tips of leaves, and numbers of trichomes on their adaxial and abaxial surfaces were counted under a binocular microscope. Latex exudation was sampled in the field by cutting the tips (0.5 cm) off intact leaves (24 total per plant type), collecting the exuding latex into pre-weighed tubes with a filter paper wick, and weighing the samples on a microbalance.

Asclepias tuberosa ‘Blonde Bombshell’ was not included in this experiment because of poor regeneration in 2019 and market unavailability.

Six additional leaves from each milkweed type were collected from separate, mature plants in July 2018 and stored at -80°C. The samples were lyophilized, then in February 2019 they were taken to the laboratory of Dr. Stephen Malcolm (Western Michigan University, Kalamazoo, MI) for cardenolide analysis using methods of Wiegrebe and Wichtl (1993) and Malcolm et. al. (1989). Briefly, the samples were extracted in methanol, centrifuged, washed in methanol, and dried in a nitrogen evaporator at 60°C. Dried extracts were re-suspended in acetonitrile and filtered through a 0.45 µm luer-lock syringe filter into a 1 ml autosampler vial ready for HPLC analysis. Samples analyses were performed on a Waters gradient HPLC system with WISP autosampler, 600E pump, 996 diode array detector and Millennium® chromatography software. Cardenolides were detected at 218.5 nm and identified by their symmetrical spectra between 205 and 235 nm and a λmax of between 214 and 224 nm. Cardenolide concentration for each peak (µg/0.1g sample DW) was calculated from a calibration curve with the external cardenolide standard digitoxin (Sigma, St Louis, Missouri). Only cardenolide peaks reported by Millennium software as consistently pure were considered for analysis.

In addition, mature greenhouse-grown potted plants of all non-native milkweed types (*A. curassavica* wild type and cultivars; *G. physocarpus*) were tested for latex exudation and trichrome density in June 2019. Methodology was as described above.

Plant Characteristics

Physical characteristics of each wild-type and cultivar (both native and non-native) were visually assessed (Table 4.1). Bloom period, plant height, and canopy width were assessed in the field for each milkweed type in gardens. Measurements were taken after bloom when plants had reached maturity.

Bee and butterfly assemblages of garden milkweeds

We collected samples of 50 or more bees from blooms of at least four, and in most cases six, replicates of each milkweed type. Some milkweeds, e.g., *A. incarnata* wild type, and *A. tuberosa* ‘Hello Yellow”, bloomed sparsely in one or two plots which limited the sample size that could be obtained from those gardens. Bees were collected using aerial nets or by knocking them into plastic containers containing 70% EtOH on multiple visits during peak bloom in 2018 and 2019. Bee samples were washed with water and dish soap, rinsed, then dried using a fan–powered dryer for 30–60 min. The pinned specimens were identified to genus (Packer et. al 2007) and honey bees and bumble bees were taken to species (Williams 2004).

We also collected at least butterflies nectaring on the milkweeds during bloom for general comparison. Specimens were mounted, familiar species were spot-identified, and others were or identified using (Iftner et. al. 1992).

Statistical analyses

Monarch colonization, larval performance, defensive characteristics, and plant characteristics were compared among milkweed groups by two-way analysis of variance

(ANOVA) for a randomized complete block design and reported as means ± standard error (SE). Two-tailed Dunnett's tests were used to compare means between milkweed cultivars and their respective wild types.

Bee genus richness and diversity (Simpson 1-D; Magurran 2004) were compared within milkweed groups by ANOVA for a randomized complete block. Statistical analyses were performed with Statistix 10 (Analytical Software 2013). Data are reported as original (non-transformed) means ± standard error (SE).

Results

Monarch colonization

All of the gardens attracted monarchs throughout the 2018 and 2019 growing seasons (238 and 207 respectively). Significantly more monarch eggs and larvae were found on *A. incarnata* than *A. tuberosa* in 2018 ($F_{7,47} = 5.25$, $P < 0.001$) and 2019 ($F_{6,41} = 6.29$, $P < 0.001$). We observed no differences in colonization between *A. incarnata* wild-type and its cultivars in either year (2018 $F_{3,15} = 0.8$, $P = 0.51$; 2019 $F_{3,15} = 1.08$, $P = 0.39$) (Figure 4.2). Monarchs on *A. incarnata* were first observed in June 2018 and persisted throughout September, peaking in August. In 2019, the first monarch progeny were recorded in May and peaked in August with similar trends for *A. tuberosa* wild-type and its cultivars. There were no differences between *A. tuberosa* wild-type and its cultivars in either year (2018 $F_{3,15} = 1.33$, $P = 0.30$; 2019 $F_{3,15} = 0.35$, $P = 0.71$) (Figure 4.3). *Asclepias tuberosa* 'Blonde Bombshell' was not included in 2019 due to poor regeneration of plants and market unavailability.

Larval performance

Monarch larvae grew and developed on all milkweeds tested. Larval growth and development was similar between *A. incarnata* wild-type and its cultivars (Figure 4.4A,B) ($F_{3,24}$ = 0.14, P = 0.94; $F_{3,24}$ = 0.52, P = 0.67 respectively). There also were no differences between *A. tuberosa* and its cultivars in either trial (Figure 4.5A,B) ($F_{3,15}$ = 3.20, P = 0.07 $F_{3,15}$ = 1.55, P = 0.24 respectively). Although not significant, larvae tended to grow more slowly on cultivar 'Hello Yellow' than on wild-type *A. tuberosa*. Larval weight gain was similar on *A. curassavica* wild-type and its cultivars $F_{3,18}$ = 1.58, P = 0.23 (Figure 4.6A), but instar achieved was less on 'Silky Deep Red' compared to the wild-type (Figure 4.6B).

Defensive characteristics

Expression of defensive characteristics varied among milkweed types (Table 4.2). Within the *A. incarnata* group, 'Cinderella' had significantly higher latex expression than the wild-type. 'Ice Ballet' had similar latex expression, but significantly more trichomes and higher cardenolide concentrations compared to the wild-type and other cultivars. In the *A. tuberosa* group 'Gay Butterflies' and 'Hello Yellow' had significantly higher latex expression than the wild-type. Interestingly, *A. curassavica* wild-type had significantly higher latex expression than any of its cultivars. Monarch caterpillar mortality was similar among all milkweed groups similar ($F_{11,99}$ = 1.38, P = 0.19), suggesting that differences in expression of defensive characteristics in milkweeds due to cultivation are not severe enough to influence survival.

Plant characteristics

Plant stature was very similar within the *A. incarnata* group, with exception to 'Soulmate' which had a larger canopy (Table 4.3). The *A. tuberosa* cultivars 'Gay Butterflies' and 'Hello Yellow' tended to be larger in both height and canopy width than the wild-type.

Bee and butterfly assemblages of garden milkweeds

Bee genus diversity was similar within the *A. incarnata* group ($F_{3,15} = 1.74$, $P = 0.2$) (Table 4.4). Among *A. tuberosa* types, 'Blonde Bombshell' had significantly lower genus diversity than the wild-type ($F_{3,15} = 5.82$, $P = 0.007$) despite that cultivar attracting a relatively large number of genera. Most of the bees collected from 'Blonde Bombshell' were Halictidae, genus *Lasioglossum* (71%). Bee assemblages of *A. incarnata* were dominated by apid bees, particularly *Bombus* and *Xylocopa* spp. and *A. mellifera*, whereas those of *A. tuberosa* had a somewhat more even distribution of families and genera, with proportionately more Halictidae (Figures 4.7, 4.8).

Within milkweed species, wild type plants and nativars attracted generally similar butterfly and moth assemblages (Figure 4.9). Proportionate abundance of particular families varied, but Erbidae seemed to favor *A. incarnata* 'Cinderella' and the white-flowered 'Ice Ballet' over the other milkweed types.

Discussion

Many of the plants available for purchase at garden centers are horticultural selections with varying degrees of attractiveness to pollinators (Corbet 2001; Garbuzov et al. 2015, 2017). The limited number of studies addressing effects of horticultural selection on insects mainly focus on whether the modified plants still support pollinators, not host plant suitability for pests or charismatic leaf-feeders such as butterfly larvae (Wilde et al. 2015). As nativars become more readily available and are marketed and sold for use in conservation gardens the need for such research increases. In one example, the ninebark beetle *Calligrapha spireaeae* (Coleoptera: Chrysomelidae) responds differently in both feeding and oviposition on cultivars of its host plant with varying ornamental traits such as leaf color (Tencazar and Krischik 2007). Selecting for ornamental characteristics can influence defensive characteristics in plants, in the case of the ninebark beetle, the least attractive plant had higher concentrations of defensive compounds and reduced nitrogen in the leaves (Tencazar and Krischik 2007).

Shared evolutionary history of insects and host plants has led to specialization. Lepidoptera often have limited host ranges and are generally restricted to a single genus on which they deposit their eggs and rear larvae (Dyer et al. 2007). Manipulation of plant characteristics may influence the pathways that lead specialist herbivores to accept a host. Host acceptance in monarchs has been suggested to be driven by compounds such as flavanol glycosides (Haribal and Renwick 1998), cardenolide content (Zalucki et al. 1990), and nutrient (e.g., nitrogen) content of the foliage. When a female monarch encounters a host plant, the path to acceptance is influenced by sensillae located on the antennae, forelegs, and midlegs (Haribal and Renwick 1998). Changes in the expression of chemical cues may be altered by horticultural selection and may change the perception of its insect associates. Further, insects that are using combination of visual and chemical ques may not be able to recognize a host plant with altered leaf color or shape as readily as a wild-type plant.

Restoring enough milkweed to rectify monarch habitat lost due to agricultural intensification and urbanization is projected to require participation by all land use sectors including metropolitan areas (Thogmartin et al. 2017b; Johnston et al. 2019). For the urban sector's contribution to that 'all hands on deck' (Thogmartin et al. 2017b) to truly benefit monarch conservation, the milkweeds planted in urban gardens must be acceptable for oviposition and support larval development and survival at levels comparable to milkweeds in more rural or natural settings. Otherwise, urban gardens could become ecological sinks or traps (e.g., Levy and Connor 2004) by luring ovipositing females away from better quality habitat. Another potentially negative scenario would be if nativars incur comparable or higher egg-loading than do wild-type plants, but because of altered timing of plant senescence or other differences, cause the monarchs to have altered behavior or greater exposure to natural enemies, such as occurs with easily-cultivated and widely-marketed Mexican milkweed, *A. curassavica,* a suitable larval host whose delayed senescence in late summer may "fool" monarchs into failing to migrate while exposing them to lethal protozoan pathogens that accumulate on the non-senescent plants (Satterfield et al. 2018).

Our results, however, indicate that, at least in small urban gardens, milkweed nativars are as attractive and suitable for monarchs as their congeneric wild-type or "straight" species. We saw no marked phenological differences in their bloom times or senescence, and within species, nativars and wild-types had similar defensive characteristics (trichomes, latex, and cardenolides) and supported comparable larval growth and development. Moreover, nativars, in general, attracted bee assemblages similar to those of their respective wild-type plants. Although we did not quantify relative bee attractiveness, comparison of which would have been confounded by differences in plant height, bloom area and extent of blooming, and some phenological differences in bloom time, it was obvious that some nativars, e.g., *A. incarnata* 'Soulmate', 'Cinderella', and 'Ice Ballet', were even more bee-attractive than the wild-type, probably due to their having been selected for large showy blooms.

Some butterflies, e.g., pipevine swallowtail (*Battus philenor*) form a visual search image that facilitates more efficient host-finding in the field (Rausher 1978). The relative extent to which monarchs use vision or olfaction to locate milkweeds in the field is unclear (Zalucki et al. 2016), but the fact that caged, lab-reared monarchs learned to associate the color and shape of artificial flowers with a nectar reward in laboratory trials (Cepero et al. 2015) suggests that visual cues are important. It is interesting, then, that in the gardens, we found just as many eggs and larvae on strikingly white-flowered *A. incarnata* 'Ice Ballet' as on pink-flowered wild-type swamp milkweeds. Because of its novelty, 'Ice Ballet' is quite popular with growers and consumers (L. Baker, *pers. comm.*), so it is fortunate that monarchs do not seem to discriminate against it on the basis of color. Perhaps the butterflies are attracted to it first as a nectar source, recognizing its suitability as a host plant via chemotactic and gustatory cues after landing (Renwick and Chew 1994; Bauer et al. 1998).

Nativars and other cultivars are selected for reduced genetic diversity so they are probably not appropriate for use in habitat restoration within natural areas or other settings where maintaining a reservoir of genetic variability is important for plant population resilience in a variable environment. Nativars, nevertheless, are attractive to consumers because of their novelty and aesthetics, and can therefore help reconcile the native-only plant movement with the real-world marketing aims of plant breeders, nurseries and garden centers, and consumer-driven ornamental horticulture and gardening. This study suggests that, at least in small urban pollinator gardens, milkweed nativars can have equivalent conservation value as wild-type straight species for monarchs and bees. For urban gardens, planting several species of native milkweeds, regardless of whether they are wild-type or nativars, plus a variety other plants to provide nectar and pollen throughout the growing season, is likely the best strategy for helping to support monarchs, bees, and other pollinators.

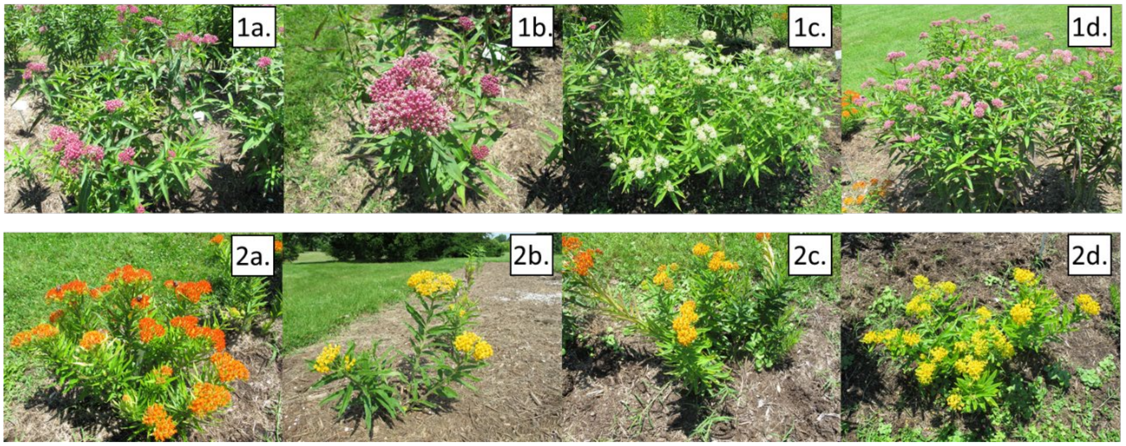

Figure 4.1 Wild-type and cultivated milkweeds as they appeared in the field in 2019. Row 1 *Asclepias incarnata*: (1a) *A. incarnata* wild-type, (1b) ‘Cinderella’, (1c) ‘Ice Ballet’, (1d) ‘Soulmate’. Row 2 *Asclepias tuberosa*: (2a) *A. tuberosa* wild-type, (2b) ‘Blonde Bombshell’, (2c) ‘Gay Butterflies’, (2d) ‘Hello Yellow’.

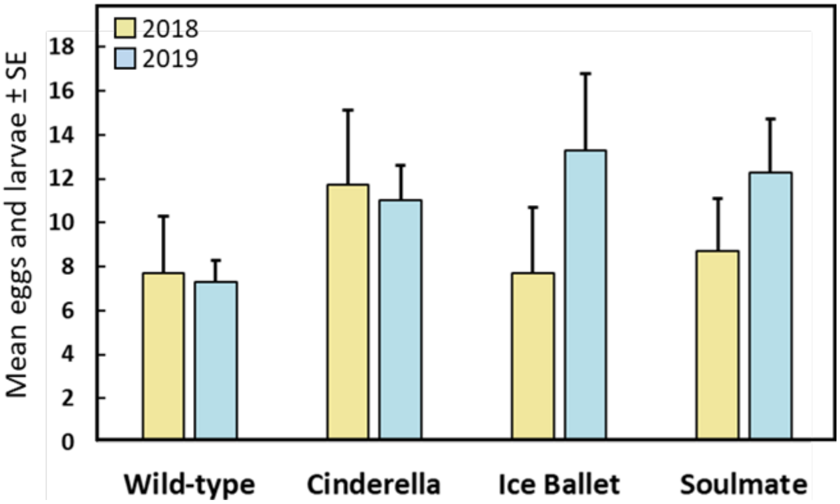

Figure 4.2 Means (SE) monarch eggs and larvae per garden for *Asclepias incarnata* wild-type and its cultivars in the 2018 ($F_{3,15} = 0.8$, $P = 0.51$) and 2019 ($F_{3,15} = 1.08$, $P = 0.39$) growing seasons.

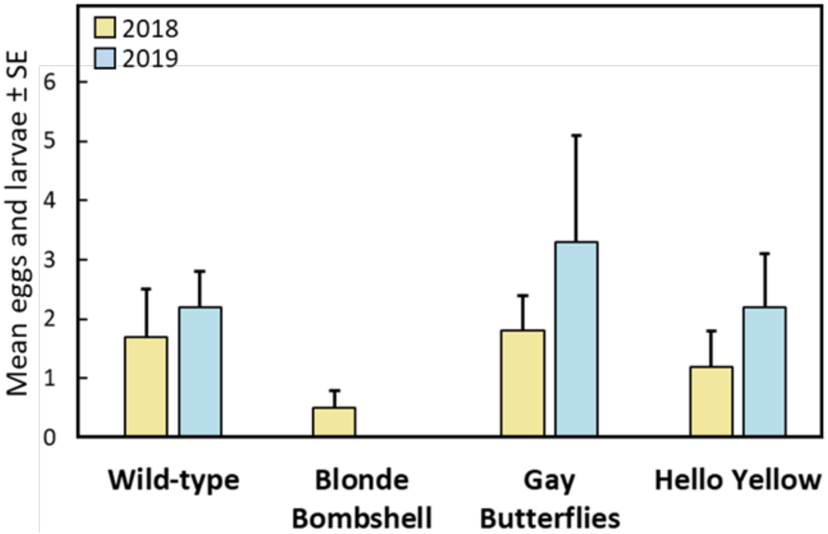

Figure 4.3 Means (± SE) monarch eggs and larvae per garden for *Asclepias tuberosa* wild-type and its cultivars in the 2018 ($F_{3,15} = 1.33$, $P = 0.30$) and 2019 ($F_{3,15} = 0.35$, $P = 0.71$) growing seasons. Due to poor regeneration 'Blonde Bombshell' was not included in 2019.

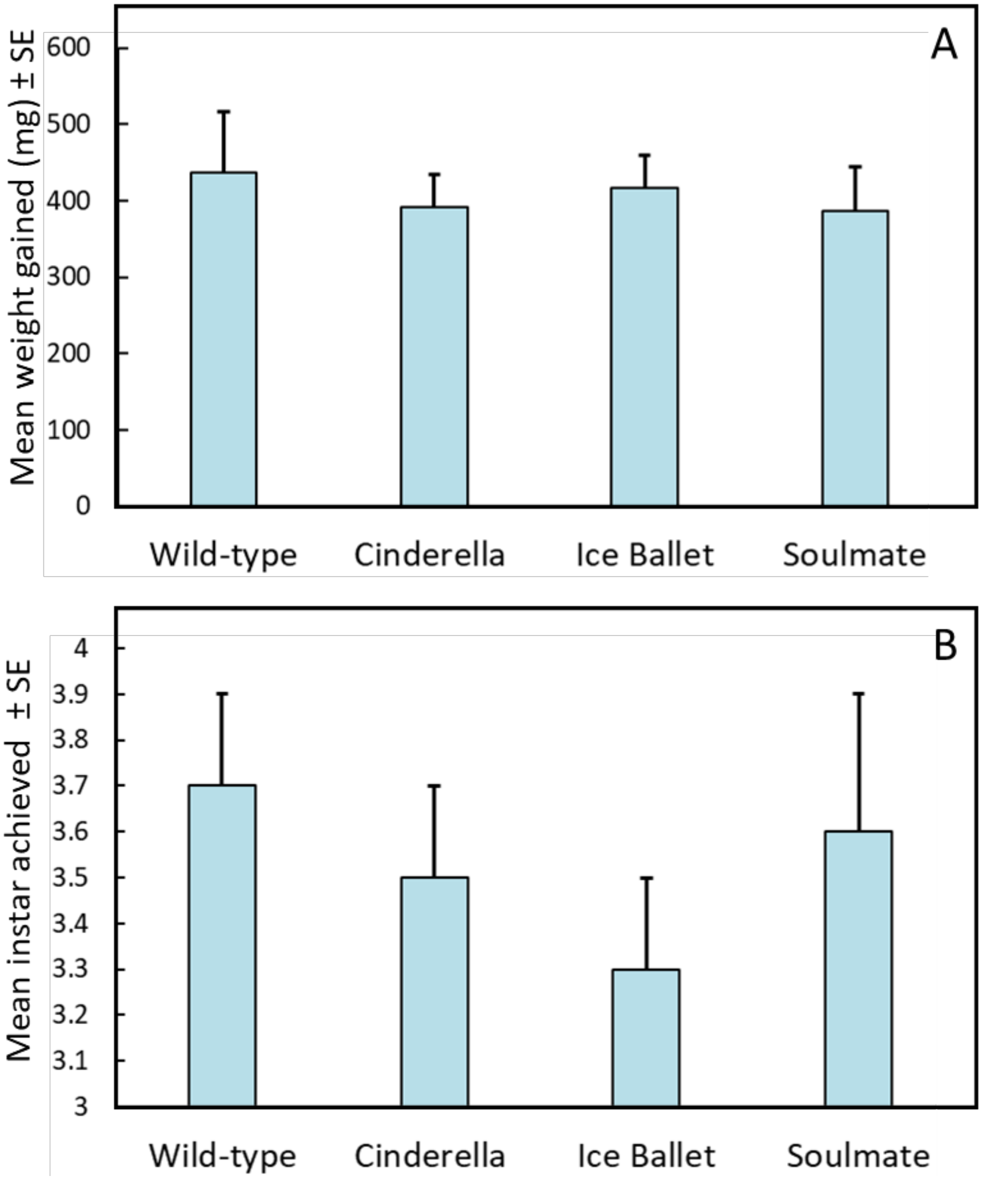

Figure 4.4 Summary data for 7d monarch larvae rearing trial on *Asclepias incarnata* wild-type and its cultivars. (A) Means (± SE) for weight (mg) gained ($F_{3,24} = 0.14$, $P = 0.94$). (B) Means (± SE) for instar achieved ($F_{3,24} = 0.52$, $P = 0.67$).

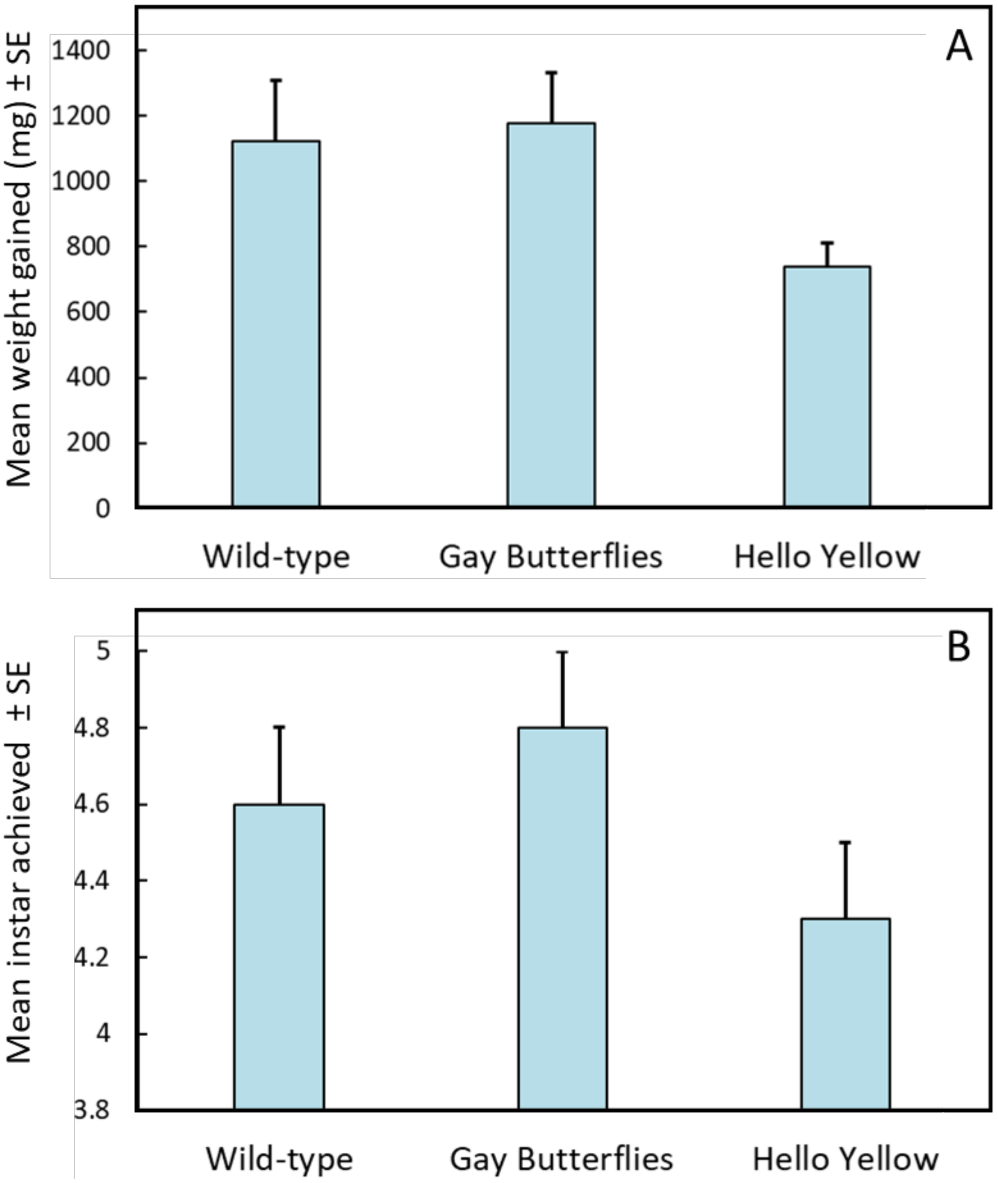

Figure 4.5 Summary data for 7d monarch larvae rearing trial on *Asclepias tuberosa* wild-type and its cultivars. (A) Means (± SE) for weight (mg) gained ($F_{3,15} = 3.20$, $P = 0.07$). (B) Means (± SE) for instar achieved ($F_{3,15} = 1.55$, $P = 0.24$). Due to poor regeneration 'Blonde Bombshell' was not included in this trial.

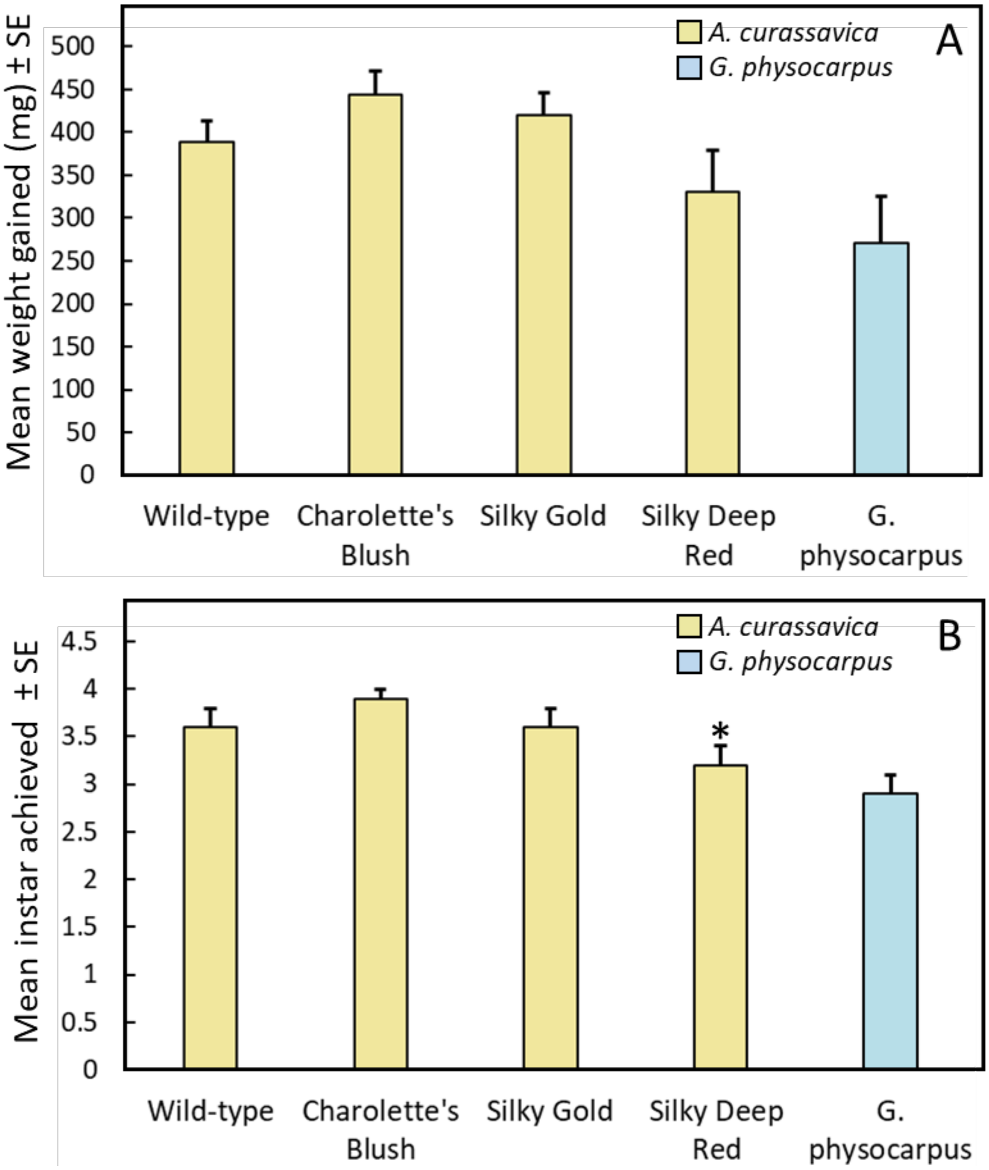

Figure 4.6 Summary data for 7d rearing trial for *Asclepias curassavica* wild-type and its cultivars and *Gomphocarpus physocarpus*. (A) Means (± SE) for weight (mg) gained ($F_{3,18} = 1.58$, $P = 0.23$ within *A. curassavica*). (B) Means (± SE) for instar achieved ($F_{3,15} = 3.69$, $P = 0.03$). * denotes significant difference from wild-type within species by 2-tail t-test.

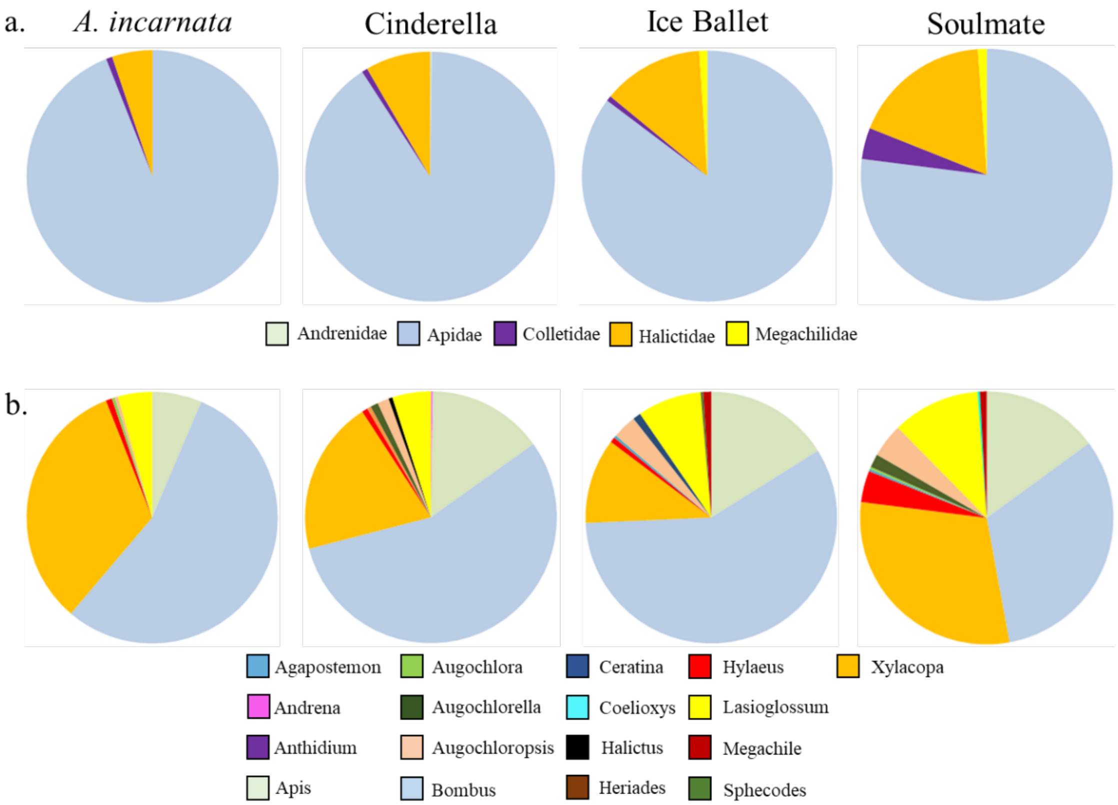

Figure 4.7 Bee assemblages of *A. incarnata* wild-type and its cultivars. a.) Bees of *A. incarnata* group by family. b.) Bees of *A. incarnata* group by genus.

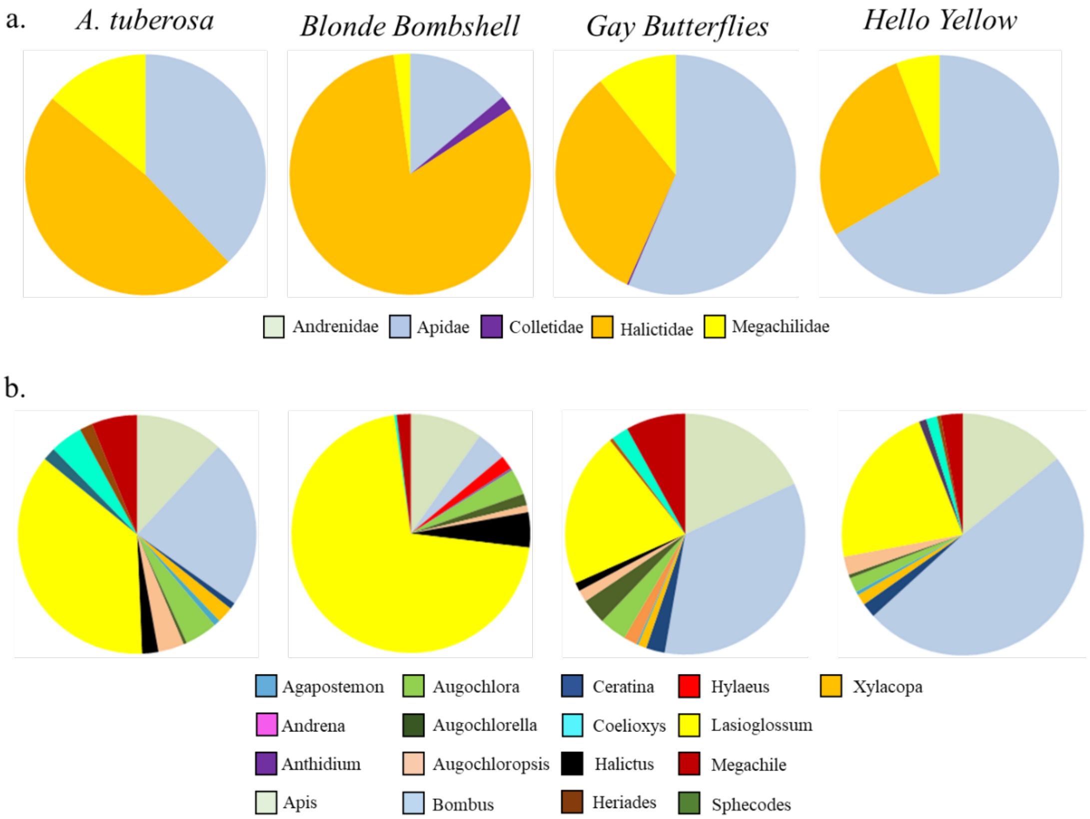

Figure 4.8 Bee assemblages of *A. tuberosa* wild-type and its cultivars. a.) Bees of *A. incarnata* group by family. b.) Bees of *A. tuberosa* group by genus.

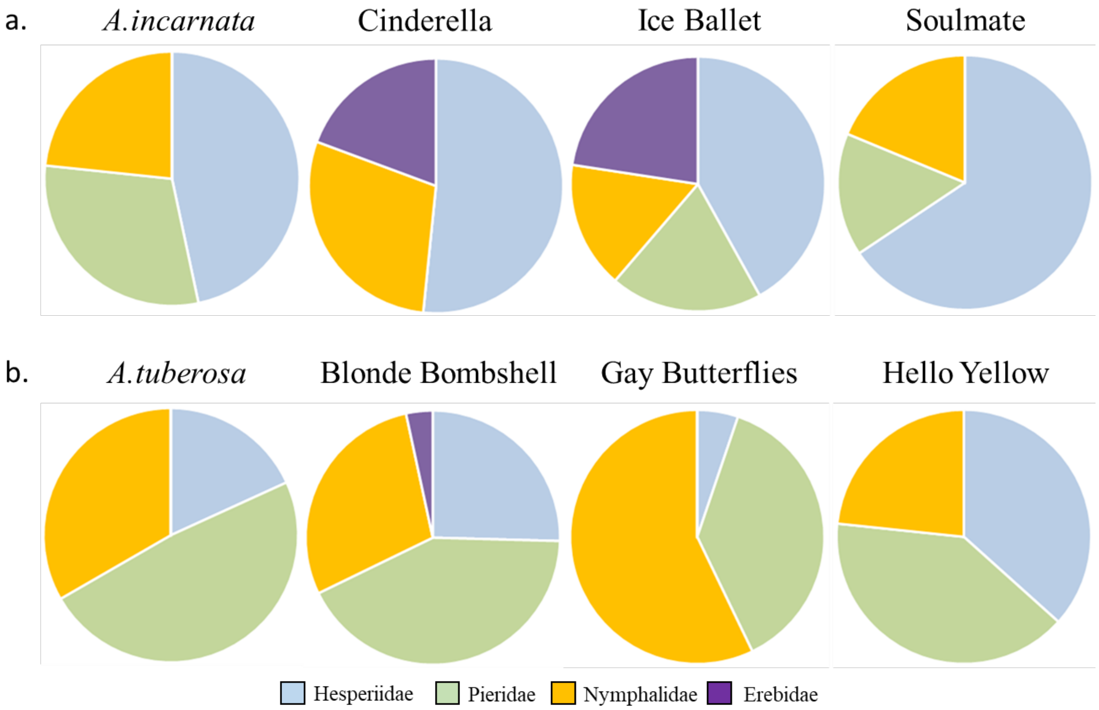

Figure 4.9 Butterfly and moth assemblages of wild-type and cultivated milkweeds by family. a.) *A. incarnata* wild-type and its cultivars. b.) *A. tuberosa* wild-type and its cultivars.

Table 4.1 Ornamental characteristics of milkweed cultivars

	Bloom color	Foliage color	Additional features
Natives			
A. incarnata			
Wild-type	pink	kelly green	--------------
Cinderella	darker pink	kelly green	larger flower clusters
Ice Ballet	white	light green	shorter stature
Soulmate	pink	kelly green	more flower clusters
A. tuberosa			
Wild-type	orange	dark green	--------------
Blonde Bombshell	pale yellow	medium green	--------------
Gay Butterflies	red, orange, yellow	medium green	multiple bloom colors
Hello Yellow	bright yellow	medium green	larger stature
Non-natives			
A. curassavica			
Wild-type	orange and yellow	kelly green	--------
Charlotte's Blush	orange and yellow	pink, white, green	variegated leaves
Silky Gold	yellow	pale green	--------------
Silky Deep Red	dark red and orange	green with red tint	--------------
G. physocarpus			
Wild-type	white	pale green	--------------

Table 4.2 Defensive characteristics of wild-type and cultivated milkweeds

	Latex (mg exuded)	Trichomes per 28 mm^2	Cardenolides (µg/g)
Natives			
A. incarnata			
Wild-type	1.4 ± 0.2	97 ± 13	4.6 ± 1.8
Cinderella	3.4 ± 0.8*	93 ± 14	4.9 ± 2.8
Ice Ballet	1.1 ± 0.2	131 ± 13*	18.5 ± 6.3*
Soulmate	1.1 ± 0.2	92 ± 14	12.2 ± 3.4
F	$F_{3,35} = 11.22$	$F_{3,67} = 3.07$	$F_{3,15} = 2.33$
P	$P = < 0.001$	$P = 0.03$	$P = 0.01$
A. tuberosa			
Wild-type	0.7 ± 0.2	212 ± 17	392 ± 93
Blonde Bombshell	----------	----------	489 ± 148
Gay Butterflies	2.1 ± 0.4*	202 ± 27	684 ± 535
Hello Yellow	2.3 ± 0.3*	153 ± 21	498 ± 296
F	$F_{2,31} = 14.36$	$F_{2,64} = 2.62$	$F_{3,14} = 0.25$
P	$P < 0.001$	$P = 0.08$	$P = 0.86$
Non-natives			
A. curassavica			
Wild-type	3.6 ± 0.4	50 ± 11	----------
Charloette's Blush	1.5 ± 0.1*	37 ± 9	----------
Silky Gold	1.4 ± 0.2*	61 ± 6	----------
Silky Deep Red	1.8 ± 0.2*	71 ± 10	----------
F	$F_{3,85} = 14.31$	$F_{3,33} = 3.92$	----------
P	$P < 0.001$	$P = 0.02$	----------
G. physocarpus			
Wild-type	2.6 ± 0.3	141 ± 24	----------

* denote significant difference from wild-type

Table 4.3 Plant characteristics of milkweeds in the gardens

	Mean height (cm)	Mean canopy width (cm)	Bloom period
A. incarnata			
Wild-type	89 ± 5.3	68.4 ± 5.6	June-July
Cinderella	91 ± 5.6	77.9 ± 3.2	June-July
Ice Ballet	77.5 ± 4.2	77.8 ± 6.1	June-July
Soulmate	99.3 ± 1.7	95.3 ± 4.8*	June-July
A. tuberosa			
Wild-type	32.5 ± 0.7	36.6 ± 1.1	June-July
Gay Butterflies	48.1 ± 2.3*	58.3 ± 2.4*	June-July
Hello Yellow	45.6 ± 1.9*	51.1 ± 2.6*	June-July

* denotes significant difference compared to wild-type within species, ANOVA, 2-tailed Dunnett's test, $P < 0.001$

Table 4.4 Bee assemblages of cultivated and wild-type milkweeds

	A.incarnata				*A. tuberosa*			
	Wild-type	Cinderella	Ice Ballet	Soulmate	Wild-type	Blonde Bombshell	Gay Butterflies	Hello Yellow
Andrenidae								
Andrena sp.	0	1	0	0	0	0	0	0
Apidae								
Apis mellifera	16	60	47	52	27	31	79	29
Bombus bimaculatus	0	12	0	2	6	1	5	9
B. griseocollis	137	213	165	110	41	9	117	75
B. impatians	0	1	5	0	4	3	29	16
B. pensylvanicus	0	0	0	1	0	0	0	0
Ceratina sp.	0	0	0	0	2	0	11	4
Xylocopa virginica	82	80	32	104	5	0	5	3
Colletidae								
Hylaeus sp.	2	3	2	14	0	6	1	0
Halictidae								
Agapostemon sp.	0	2	1	1	2	1	8	1
Augochlora sp.	1	0	0	1	10	11	16	4
Augochlorella sp.	0	4	0	6	1	5	15	1
Augochloropsis sp.	1	6	9	15	8	3	7	5
Halictus sp.	0	2	3	0	5	15	5	0
Lasioglossum sp.	11	20	24	39	83	224	91	45
Sphecodes sp.	0	0	1	0	0	0	0	0
Megachilidae								
Anthidium sp.	0	0	0	0	4	0	2	2
Coelioxys sp.	0	0	0	1	10	1	10	3
Heriades sp.	0	0	0	0	4	0	0	1
Megachile sp.	0	0	3	3	14	6	35	6
Replicates	6	6	6	6	6	6	6	6
Total Bees Sampled	250	404	291	346	227	317	398	203
Genus Richness	5	7	8	9	10	11	8	13
Genus Diversity	0.59 ± 0.04	0.61 ± 0.08	0.63 ± 0.03	0.74 ± 0.04	0.74 ± 0.11	0.46 ± 0.07	0.75 ± 0.02	0.83 ± 0.02

CHAPTER 5

Invasive paper wasps turn urban monarch butterfly conservation gardens into ecological traps

Introduction

Invasive species can be particularly disrupting when they intersect with organisms of conservation concern (Dueñas et al. 2018). Urban ecological restoration can sometimes facilitate ecological traps by luring native species to colonize patches of semi-natural habitat where they incur inordinately high mortality from exotic natural enemies (Robertson et al. 2013; Lepczyk et al. 2017). For example, songbirds drawn to naturalized suburban habitat for nesting may suffer heavy predation by (non-native) domestic cats (Loss et al. 2012; Shipley et al. 2013). Urbanization itself can magnify such interactions by providing nesting sites or other resources for synanthropic invasive predators (Schlaepfer et al. 2005; Shochat et al. 2010; Fletcher et al. 2012). As urban citizens increasingly plant gardens to support native pollinators and other biodiversity (Goddard et al. 2010; Lepczyk et al. 2017), it is important those efforts do not inadvertently create ecological traps for species they are intended to benefit.

Populations of the monarch (*Danaus plexippus*), an iconic migratory North American butterfly, are declining (Brower et al. 2012; Vidal et al. 2014) and conservationists are encouraging planting milkweeds (*Asclepias* spp.), the monarch's obligate larval host plants, to help offset habitat loss across the breeding range (Thogmartin et al. 2017). Despite the public's enthusiasm for monarch-friendly gardening (Monarch Watch 2020; Monarch Joint Venture 2020), and projections that restoring enough milkweed to ensure a stable monarch population will require participation by the urban sector (Thogmartin et al. 2017; Johnston et al. 2019), the conservation value of urban milkweed gardens remains uncertain. Such gardens attract ovipositing adults, often with higher egg-loading per plant than occurs in natural milkweed stands (Cutting and Tallamy 2015; Stenoien et al. 2015; Baker and Potter 2018, 2019; Geest et al. 2019), but they could also become ecological traps if they by expose monarchs to increased risk of predation, disease, or abiotic mortality factors.

Polistes dominula, or European paper wasp (EPW), was first reported in North America in the 1970s where it has since become widespread (Cervo et al. 2000; Leibert et al. 2006). This wasp species' strong proclivity to nest in sheltered places associated with buildings and other structures contributes to its invasion success in urban settings (Höcherl and Tautz 2015), as does its strategy of forming nests with multiple, often unrelated, foundresses that results in high nest survival and provides a competitive edge over sympatric native paper wasps (Cervo et al. 2000; Liebert et al. 2006). Paper wasps prey on soft-bodied arthropods that they find by hovering over or walking on plants (Raveret Richter 2000; Rayor 2004). Victims are killed by biting, masticated to a manageable size, flown back to the nest either whole or piecemeal, and fed to the wasps' developing larvae (Raveret Richter 2000; Rayor 2004). Although EPW are opportunistic, generalist predators, individuals often return repeatedly to hunt in sites of previous hunting success (Raveret Richer 2000). Although the wasps do not actively recruit nest mates, they are attracted to other individuals' inspection or processing of prey (Rayor 2004, authors' observations). In a greenhouse study, EPW preyed on monarch larvae regardless of cardenolide concentrations found in the milkweed species upon which the larvae had fed (Rayor 2004).

During field studies aimed at enhancing monarch colonization of urban pollinator gardens (Baker and Potter 2018, 2019) we observed EPW attacking monarch larvae. Paper wasp predation has not previously been studied in the context of monarch conservation gardens, but given EPW's synathropy (Liebert et al. 2006) we hypothesized it may pose particular danger to monarch larvae in urban settings. Here, we verify that EPW is the predominant paper wasp foraging in urban gardens in central Kentucky, document higher *Polistes* predation on monarchs in urban gardens compared to more rural settings, and describe behavior and fate of monarchs attacked by EPW in such gardens. We also show that "butterfly hibernation boxes" (Johnson 2019) in flower gardens are exploited by EPW as nesting habitat. Our findings identify EPW as a previously under-recognized mortality factor that can turn urban milkweed gardens into ecological traps for monarch larvae and potentially diminish the urban sector’s contributions to monarch habitat restoration.

Methods and Materials

Assessing EPW prevalence in urban gardens.

Sixteen pre-existing urban pollinator gardens at residences, campuses, and businesses within the Lexington, Kentucky city limits were monitored for presence of foraging paper wasps. Observations took place throughout July 2019, on afternoons (1200–1700 h) of clear warm (> 25° C) days. Each garden was visited once by two independent observers who focused on different portions of the garden for 30 min, recording numbers of wasp visits to each garden. Wasps exhibiting predatory searching behavior were counted; those nectaring at flowers were not. Wasps were tracked from the time they entered the garden until they left the garden and surrounding area. All of the gardens had unique features, but all were close to buildings, of similar size, and contained a mixture of flowering herbaceous plants.

EPW encounters with monarch caterpillars in gardens.

We recorded outcomes of 120 encounters (30 per instar 2nd -5th) between wild EPW foragers and monarch larvae feeding on mature swamp milkweed (*Asclepias incarnata*) in outdoor urban garden settings. The milkweeds were grown from 2-yr old rootstock in a soil/bark mix (Sun Gro, Quincy, MI) in 5.6 liter pots and about 90 cm tall when used. Observations took place from 7–31 July at three pre-existing urban pollinator gardens, two of them (> 300 m apart) on the University of Kentucky Lexington campus and the third at a residence about 3 km away. All gardens contained a similar mix of flowering nectar- and butterfly host-plants; e.g., milkweeds (*Asclepias* spp.), spicebush (*Lindera benzoin*), asters (*Aster* spp.), cone flowers (*Echinacea* spp.) and others. Before each observation period, 10 monarch larvae were placed on separate leaves of an undamaged swamp milkweed and allowed to establish for about 1 h. The plant was then placed in a garden and watched continuously for 90 min. All observations were on clear warm (> 25°C) sunny days between 1100–1700 h, from 7 July to 1 August. Larvae taken during a given observation period were not replaced. Fresh plants and larvae were used for each observation period.

Predation on monarch larvae in urban and rural settings.

Twenty mature swamp milkweeds, as above, were each seeded with cohorts of 10 monarch larvae (third and fourth instars) that were secured, five each on abaxial or adaxial leaf surfaces, by inserting a fine insect pin through the anal prolegs and leaf into a bit of cork on the opposite side. As a check for possibility of escapes, 30 larvae were similarly affixed to plants in the greenhouse, where 100% were still in place after 8 h.

Plants with sentinel larvae were placed in 10 urban gardens where EPW had been observed, and in open meadow habitat at 10 rural sites, left in the field for 8 h (1100 – 1900 h), and then inspected for signs of predation. Rural sites (mostly in nature parks and farm edges) contained pasture grasses and mixed wild flowering plants, including milkweed, whereas garden sites were all within the Lexington city limits. We used satellite images and the Measure Tool feature of Google Earth Pro geospatial software (Microsoft, Palo Alto CA) to measure distance from where each plant with larvae was placed to the nearest structure.

Wasp exploitation of butterfly hibernation boxes in pollinator gardens.

We observed EPW entering and exiting butterfly boxes that a student organization had placed in six, widely-spaced pollinator gardens on the University of Kentucky Campus (Fig. 5.4). To assess the extent of colonization by paper wasps, we opened the 22 boxes in October 2019 to verify if they had been occupied, and by which species. Failed nests (< 10 cells) were not counted. Wasps were still present on nests during the survey.

Statistical analyses

Numbers of foragers of different *Polistes* spp. observed in urban gardens, relative proportions of monarch instars killed during or escaping encounters with EPW, and predation on sentinel larvae in urban gardens versus rural settings were compared by one-way analysis of variance, χ^2 test for independence, and two-sample t-tests, respectively, using Statistix 10 (Analytical Software, Tallahassee, FL).

Results

Assessing EPW prevalence in urban gardens.

EPW foragers (n = 45) were observed in 10 of the 16 urban pollinator gardens surveyed for paper wasps during July. Two native paper wasp species, *Polistes fuscatus* (n = 14) and *Polistes exclamans* (n = 1) were also observed in some gardens, but *P. dominula* was the most abundant *Polistes* spp. overall ($F_{2,15} = 7.98$, $P = < 0.01$; Fig. 5.1). No wasps were observed in three of the 16 gardens, and in three others only *P. fuscatus* was seen.

EPW encounters with monarch caterpillars in gardens.

EPW readily attacked second to fifth instar monarchs on swamp milkweed in urban pollinator gardens (Fig. 5.2a,b; Table 5.1). Relative proportions preyed upon or escaping such encounters differed among instars, as did the behavior of wasps and caterpillars (Table 5.1). Smaller larvae were far more vulnerable than fifth instars. Wasps encountering second instars mostly struck, bit, and carried off their victims intact, although some (7/30) managed to avoid predation either by dropping from the plant or on a silk strand. Nearly all predation events on third instars resulted in the wasp first excising the caterpillar’s gut which was left on the leaf, then macerating the remains into a ball and flying off with it. On one occasion the larva dropped on silk and the wasp followed the strand down and carried it off. Third instars escaping predation either dropped off the plant or on a silk strand. Fourth instar kills were gutted as above, macerated, and processed into manageable pieces, the wasp often taking multiple trips to carry them back to the nest. On several occasions, we observed other wasps trying to steal prey pieces while the original wasp was still processing its kill, or to take pieces left behind. Those fourth instars escaping predation either dropped or thrashed in response to the wasp's attack. Nearly all (28/30) fifth instars escaped, either by violently thrashing or dropping. Both of the fifth instar kills were processed by multiple wasps (Fig 5.2b). In 52 h of observation, we saw no predation by natural enemies other than *P. dominula*.

Predation on monarch larvae in urban and rural settings.

Sentinel monarch larvae (third and fourth instars) exposed on swamp milkweeds placed in 10 urban pollinator gardens sustained significantly more predation than did similar cohorts placed in mixed-plant meadow habitat in rural areas (Fig. 5.3). In nearly every case, the larva's excised digestive tract was left on the plant near the pin that had secured it (Fig 5.2 c,d), indicative of predation by *Polistes* as opposed to other chewing predators (e.g. birds) that consume the entire larva, or sucking predators (e.g., stink bugs) that drain the hemolymph. Mean distance between sentinel larvae exposure sites and closest buildings were 6.5 ± 1.3 m (range 3–16 m) and 257 ± 15 (range 184–340 m) for urban gardens and rural milkweed patches, respectively ($t = 16.8$, $P < 0.001$). We observed EPW foragers in all 10 pollinator gardens.

Wasp exploitation of butterfly hibernation boxes in pollinator gardens.

Twenty two butterfly boxes (Fig 5.3a) (also called butterfly hibernation boxes) in six pollinator conservation gardens on University of Kentucky's campus were opened and inspected in autumn. Sixteen of the boxes contained *Polistes* wasp nests. Thirteen of those boxes were occupied by *P. dominula*, two by *P. fuscatus*, and one by *P. exclamans*. We saw no evidence of butterflies using the boxes, although some boxes did contain spiders or mantis ootheca.

Discussion

Paper wasps are abundant in most temperate ecosystems and exert strong selective pressure on lepidopteran larvae (Ravert Ritcher 2000). When invasive *Polistes* spp. are introduced to new areas they compete with native species for niche availability (Cervo et al. 2000; Gamboa et al. 2004; Liebert et al. 2006) and may elevate predation pressure, putting prey species at risk of population decline (Clapperton et al. 1999). Since being introduced into the eastern United States in the late 1970s, EPW has become widely established in North America (CABI 2019), especially in urban environments where the types of sheltered nesting sites it prefers are plentiful (Cervo et al. 2000; Höcherl and Tautz 2015). Although *Polistes* spp. can be efficient biocontrol agents for lepidopteran pests in urban agriculture (e.g., Gould 1984; Prezoto et al. 2019), this study highlights the potential for EPW, in particular, to decimate monarch larvae in urban gardens.

Monarchs typically incur high (90–95% or more) mortality from egg to fifth instar (Zalucki and Kitching 1982c; Prysby 2004; Oberhauser et al. 2015; De Anda and Oberhauser 2015; Nail et al. 2015). Host plant defenses account for some larval mortality, especially of early instars (Zalucki and Malcolm 1999), but invertebrate natural enemies probably account for more (Oberhauser et al. 2015). Monarch larvae may be killed and eaten by ants, spiders, predatory bugs, mantids, lady beetles, vespid wasps, or other arthropods (Zalucki and Kitching 1982c; Oberhauser at el. 2015; Hermann et al. 2019) or parasitized by tachinid flies (Oberhauser et al. 2017b) or chalcid wasps. While numerous studies have inferred causes of predation by tracking stage-specific disappearance of monarch eggs and larvae in the field (e.g., Zalucki and Kitching 1982c; Prysby 2004; De Anda and Oberhauser 2015; Nail et al. 2015; Oberhauser et al. 2015), few have observed and quantified predation events directly. In particular, EPW has received scant mention, mainly anecdotally, as a predator of monarch larvae in field settings.

In addition to direct predation, encounters with EPW may indirectly impact survivorship of monarch larvae by causing them to drop from the plant where they might be exposed to ground-dwelling predators, or cause larvae to feed in suboptimal microhabitats; e.g., inner or basal portions of the plant with lower temperatures or less nutritious leaves, to escape from the wasps. Indirect effects of harassment by *Polistes* spp. have been shown to significantly amplify the direct impact of predation in other systems (Stamp and Bowers 1991). We did not track movement or fate of monarch caterpillars after they dropped from plants, but such indirect effects warrant future investigation.

The one previous published study of EPW predation on monarch larvae deployed active wasp nests transplanted to a greenhouse to test the hypothesis that larvae raised on different *Asclepias* species present a spectrum of palatability (Rayor 2004). In this study Rayor observed that captive free-flying wasps took monarch larvae regardless of the cardenolide content of the milkweed species upon which they had been reared, although overall, larvae that had fed on milkweeds with relatively low cardenolide content were preferred (Rayor 2004). Notably, larvae reared on *A. incarnata*, *A. syriaca*, and *A. tuberosa*, three species commonly planted in butterfly gardens (Baker and Potter 2018, 2019), were all palatable. That study also concluded, based trials in which small, medium-sized, or large larvae were presented simultaneously, that the wasps largely ignore second through early third instars. In contrast, we observed EPW to quickly find and attack second and third instars in gardens.

When processing prey, *Polistes* spp. may use their mandibles to excise guts that contain plant material from the balled masses of prey tissue they carry back to their nests (Raveret Richter 2000; Rayor 2004). Such behavior may be selective, depending on the plant upon which the victim had fed (Rayor 2004). We witnessed such gutting behavior in > 95% of the EPW processing of kills of third and fourth instars in gardens. Similarly-excised digestive tracts left on milkweed leaves where sentinel larvae had been removed strongly implicates paper wasps, especially EPW, as the main factor accounting for the greater loss of monarch larvae exposed in urban gardens compared to rural sites. Chinese mantid, *Tenodera sinensis*, the only other invertebrate predator reported to gut monarchs before consuming them (Rafter et al. 2013), were never observed feeding on larvae in our gardens.

Butterfly hibernation boxes, typically made of wood with vertical slits intended for entry and bark lining the inside wall, are popular ornamental features in pollinator gardens and thought by some gardeners to provide overwintering habitat for certain butterfly species (Snetsinger 1997; Johnson 2019). Although there is little or no evidence that butterflies use such boxes, they are promoted in some gardening blogs and extension publications (e.g., Purdue University Extension 2019). As shown herein, however, such boxes are perfect nesting sites for EPW. Their presence is likely to increase predation on the larvae those gardens are meant benefit.

Although our study was restricted to one metropolitan area, EPW is likely to impact monarchs wherever the two species' distributions overlap. Indeed, there are numerous on-line anecdotal accounts of EPW preying on monarchs in urban settings throughout the butterfly's breeding range (e.g., Lewis 2016). Although our exposing multiple sentinel larvae per plant might have overestimated typical rates of field predation by evoking wasps' functional response, egg-loading may be > 6 times greater on milkweeds in urban gardens compared to natural stands (Cutting and Tallamy 2015; Stenoien 2015), so in gardens it is common for there to be several larvae on a given milkweed plant (Baker and Potter 2018). Our trials were in mid-summer when EPW colonies had many workers, so the wasp might have less impact on monarchs earlier in the growing season. EPW can be managed by limiting access to preferred nest sites (e.g., repairing holes in walls, caulking cracks in soffits and eaves, and screening vents and louvers), treating exposed nests with a wasp and hornet spray, or applying insecticidal dust to openings of infested voids (Jacobs 2015). Controlling the wasp may be necessary to prevent backyard milkweed gardens from becoming ecological traps.

Conclusion and Implications

Metropolitan areas provide a substantial canvas for monarch habitat restoration (Johnston et al. 2019) and their contribution may be essential to meet existing goals to increase planted milkweed by 1.8 billion stems to support monarch butterflies (Thogmartin et al. 2017). Although numerous programs encourage urban and suburban citizens to plant gardens with milkweeds (Monarch Joint Venture 2020, Monarch Watch 2020), the assumption that such efforts will help to stem declining monarch abundance caused by habitat loss is largely untested. There is some evidence that urban butterfly gardens may act as population sinks or ecological traps for certain species (e.g., the pipevine swallowtail, *Battus philenor*) by luring butterflies away from better quality habitat (Levy and Connor 2004).

Several authors have cautioned that monarch larvae in urban gardens could face increased risk pesticide exposure, disease, parasitism or predation (Majewska at al. 2018; Geest et al. 2019; Baker and Potter 2019), but the studies to date are equivocal, some finding no consistent difference in the overall low survival of subadult monarchs in residential or natural sites (Cutting and Tallamy 2015; Geest et al. 2019), and another suggesting that larval mortality risk was higher on sentinel plants placed inside garden plots than in more natural habitat away from those gardens (Majewska et al. 2018). None of those studies identified particular predators contributing to larval attrition. The present study highlights EPW as a previously under-recognized threat to monarch larvae in urban gardens. The probable impact of this wasp should be considered in estimates of the current and potential contribution of milkweed in urban areas to monarch conservation, and in recommendations about where best to focus future restoration efforts.

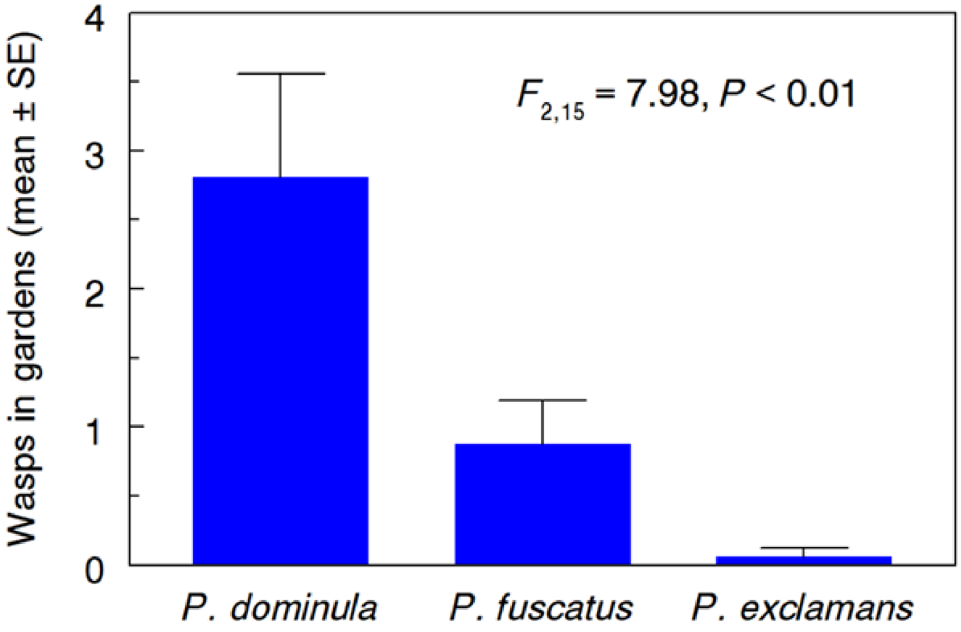

Figure 5.1 Prevalence of *P. dominula* foragers compared to other *Polistes* spp. in urban pollinator gardens based on 60 min of observation in each of 16 gardens.

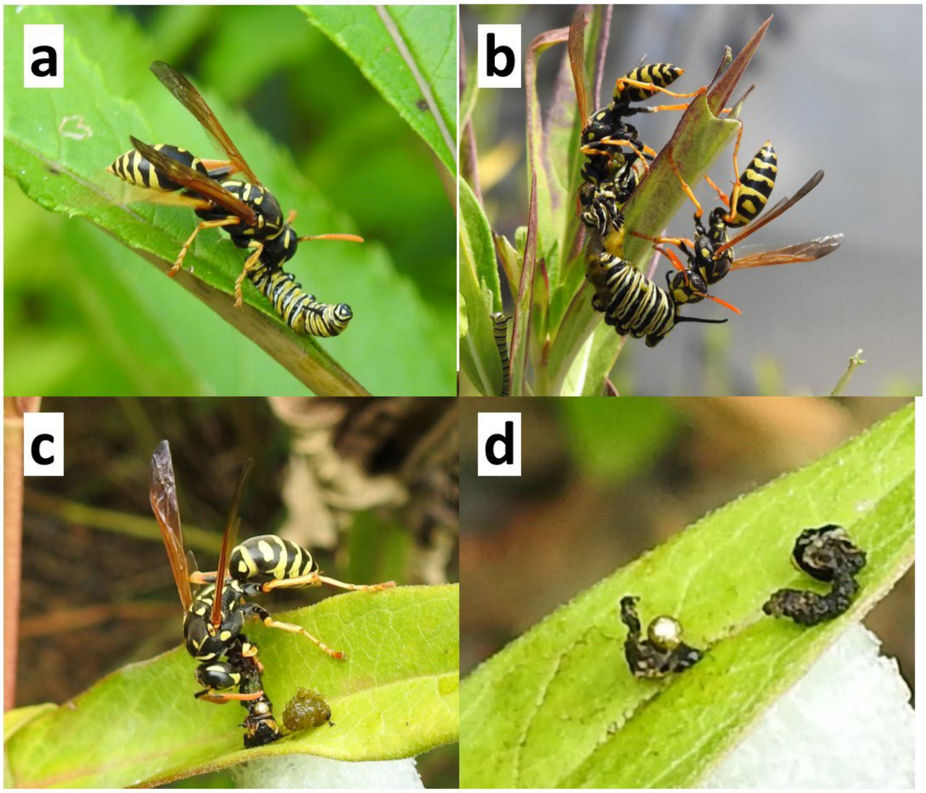

Figure 5.2 *Polistes dominula* predation on monarch larvae: a) attack on free-feeding 2nd instar, b) second wasp attracted to another's kill of free-feeding fifth instar, c) wasp gutting pinned sentinel larva, d) excised gut and head capsule indicative of *P. dominula* attack on third instar.

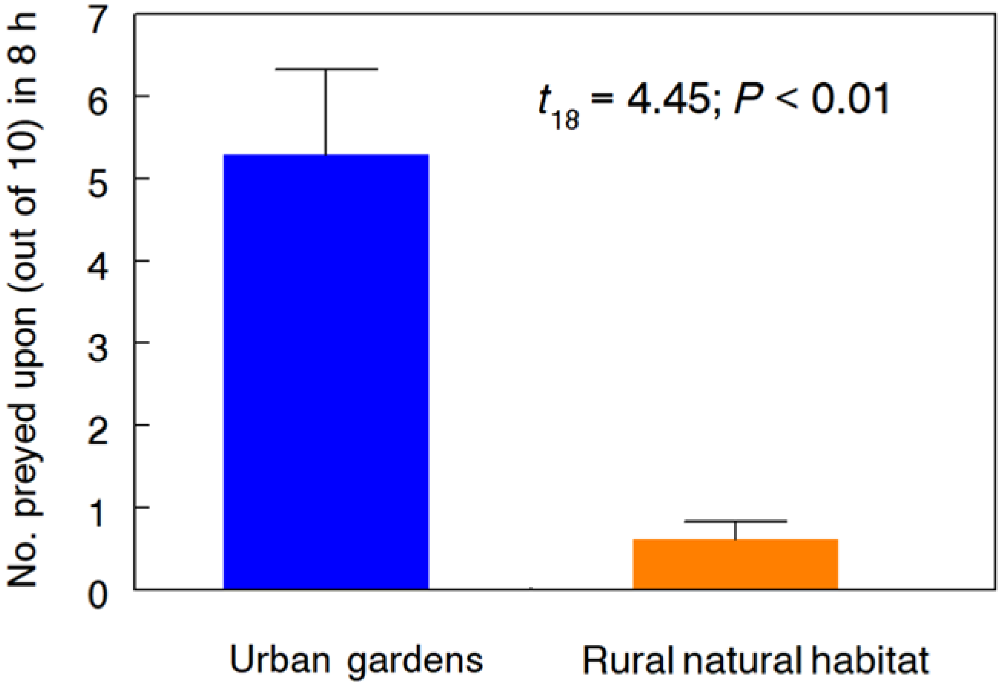

Figure 5.3 Predation of sentinel monarch larvae on swamp milkweed placed in urban pollinator gardens or patches of milkweed in rural settings. Data are means (SE) out of 10 taken after 8 h of exposure.

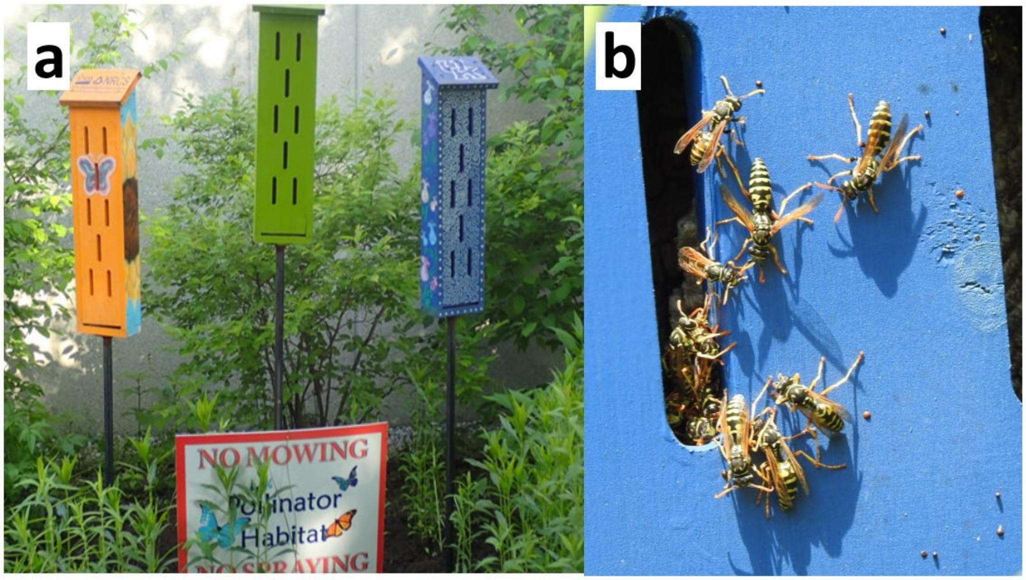

Figure 5.4 a) Butterfly boxes in urban pollinator gardens; b) Sixteen of 22 boxes in six urban pollinator gardens had been colonized by paper wasps; 13 contained active *Polistes dominula* nests.

Table 5.1 Outcome of 120 encounters (30 per instar) between *Polistes dominula* and sentinel monarch butterfly larvae feeding on swamp milkweed (*Asclepias incarnata*) plants in urban pollinator gardens

Instar	Outcome	Total[c]	Wasp kill behaviors (in sequence)[a]				Larval escape behaviors[b]		
			S,C[d]	S,G,C	S,G,P	S,W,G,P	D	DSk	T
2nd	Killed	23	21	2					
	Escaped	7					5	2	
3rd	Killed	24	2	20	2				
	Escaped	6					5	1	
4th	Killed	20		4	13	3			
	Escaped	10					5		5
5th	Killed	2				2			
	Escaped	28					8		20

[a] Wasp behaviors resulting in kill: S = strike, G = gut, C = carry off, W = wait, P = process (cut into pieces, then carry off in multiple trips)

[b] Larval behaviors leading to escape: D = drop, DSk = drop on silk, T = thrash

[c] Proportion of larvae killed or escaped differs significantly between instars (χ^2 = 43.5, df = 3, $P \leq 0.001$)

[d] includes one 2nd and one 3rd instar that dropped on silk, then was found by the wasp and carried off intact

CHAPTER 6

Japanese beetles' feeding on milkweed flowers may compromise efforts to restore monarch butterfly habitat

Introduction

The eastern migratory population of the monarch, *Danaus plexippus* L., probably the best known butterfly in the world, has declined in abundance by > 90% in the last two decades (Bower et al. 2012) and is considered at risk of extirpation (Semmens et al. 2016; Pitman et al. 2018). The monarch has become an international conservation icon with power to mobilize scientists, organizations, and the public into actions to help restore its populations, and shape environmental policy (Diffendorfer et al. 2014; Pollinator Health Task Force 2015; Gustafsson et al. 2015, 2017). Conservation of this specialist herbivore requires understanding the threats affecting its annual abundance, one of which is loss of milkweed (*Asclepias* species), the essential larval host plants, in the monarch's summer breeding grounds in the Midwestern United States (Pleasants and Oberhauser 2013; Flockhart et al. 2015; Stenoien et al. 2016; Marini and Zalucki 2017; Pleasants et al. 2017). We report here a previously undocumented biotic threat to sexual reproduction of common milkweed, *Asclepias syriaca*, which is used by > 90% of monarchs in their summer breeding range within eastern North America (Malcolm 2018; Malcolm et al. 1989, 1993; Thogmartin et al. 2017a, 2017b).

Popillia japonica Newman, commonly known as the Japanese beetle [JB], is an invasive, polyphagous scarab that was first discovered in Riverton, New Jersey, USA, near Philadelphia, in 1916 (Potter and Held 2002). Until then the species had not been known to inhabit North America. It is now widely established in the eastern United States and SE Canada, but is still expanding in abundance and range in the US Midwest (Potter and Held 2002, Center for Environmental and Research Information Systems 2018). The JB's distribution now overlaps much of geographic region that, relative to other regions, has produced the highest proportion of monarch butterflies overwintering in Mexico over the past four decades (Fig. 6.1) (Flockhart et al. 2017).

During routine surveys for monarch butterfly larvae, we observed large feeding aggregations of JB on umbels (large round inflorescences of 30–75 or more flowers) of *A. syriaca* growing wild in pasture land, naturalized areas of parks, and other settings in central Kentucky (Fig. 6.2a). The beetles were observed using their mandibles to remove the coronal hoods (saccate extensions of staminal tissue in which nectar is stored) from individual flowers to expose the nectar and other floral structures (Fig. 6.2b). Here we verify the extent of JB aggregation on milkweed and damage to umbels in wild stands of milkweed, clarify which stage of bloom and floral parts the JB prefers to feed upon, and assess the impact of JB florivory on fruit and seed set of *A. syriaca* umbels in the field.

Methods and Materials

Extent of JB infestation of *A. syriaca* in the field.

Japanese beetle [JB] florivory on wild *A. syriaca* was surveyed at two peri-urban field sites in central Kentucky, a natural-area park consisting of 133 ha of rolling pasture land (Hisle Farm Park; 38°04'27.4"N 84°23'32.7"W), and naturalized areas of a golf course, (University Club of Kentucky; 38°06'49.5"N 84°36'28.7"W), in mid-July 2017.

An additional site in naturalized areas of a different golf course (Kearney Hill Golf Links, 38°07'33.2"N 84°32'26.9"W) was sampled in early July 2018. At each site, we walked transects in four locations and scored the incidence of plants with JB aggregations or obvious severe feeding damage on their umbels. The stands of milkweed are naturally occurring at all three sites, and managed by mowing once or twice per year. In addition to milkweed, all sites contained a mix of spontaneous herbaceous plants including tall fescue (*Festuca arundinacea*), knapweed (*Centaurea* sp.), common yarrow (*Achillea millefolium*), clover (*Trifolium* spp.), poison hemlock (*Conium* maculatum), and other species resulting from natural succession into fallow areas. The sites were surrounded by areas of high-mowed (≥ 9 cm) mixed tall fescue and Kentucky bluegrass (*Poa pratensis*) and bordered by hedgerows with woody plants including black locust (*Robinia pseudoacacia*), black cherry (*Prunus serotina*), hackberry (*Celtis occidentalis*), river birch (*Betula nigra*), and sugar maple (*Acer saccharum*).

Stage of bloom and particular floral parts preferred.

Beetles were field-collected with standard JB traps (Trécé, Adair, OK, USA) baited with food-type lures (2-phenyl-ethyl-propionate, eugenol, and geraniol, 3:7:3 ratio) and brought to the lab within 4 h. Sexes were separated by foretibial characters (Fleming 1972) and males were discarded. Females were held overnight without food before each assay. Freshly caught beetles were used for each trial.

For the trial clarifying how milkweed bud development affects susceptibility to JB feeding, stems with umbels of three phenological stages (closed green bud, pink bud, or open flowers) were harvested from wild plants, placed in vases with water, and brought to the lab. Umbels were placed in 0.5 liter clear plastic containers with five females and held at 27ºC and 16:8 h (L:D) in a growth chamber for 24 h after which all flowers were excised and examined for feeding damage.

To clarify which floral organs are preferred, we harvested umbels with fully-opened flowers, separated 80 individual flowers into their component parts: coronal hoods, nectaries+ ovaries (on pedicel), or gynostegium (stigmatic chambers + pollinaria) (Wyatt and Broyles 1994) and offered to individual JB females in four-way choice tests that also included a 1-cm^2 piece of freshly-cut leaf tissue. Test arenas were translucent plastic containers (11 cm diameter, 4 cm high) with a screened lid. Feeding preference was scored after 20 min.

Impact of JB on *A. syriaca* fruit and seed set.

For trials in which JB were caged on wild plants in the field, mature umbels with beetles were enclosed in light-weight fine mesh secured around the stem using a wire twist tie (Figure 6.7). Each umbel was on a different plant. The trials were done at Hisle Farm Park (see above). The trial with manipulated JB densities used females collected with traps and starved overnight as described earlier. The JB were caged on the umbels on 26 June 2017 and removed after 24 h; the umbels were re-bagged and initial pod set was evaluated 30 d later.

For the trial with natural JB aggregations, we located non-infested umbels and ones with a range of JB densities and enclosed them in mesh as above. The plants were spaced at least 3–5 m apart to avoid disturbing the JB before they were bagged. Umbels were caged on 7 July 2017, JB were removed and counted after 24 h, and then umbels were re-bagged to prevent further florivory. Mature pods and seeds were counted on 20 September 2017.

Statistical analyses

Data were tested for assumptions of normality and homogeneity of variance implicit in parametric tests. Arcsine of square root transformation was used on percentage data. The asymptotic regression curve shown in Fig. 6.3 was fitted using an iterative function minimization algorithm (Levenberg-Marquardt-Nash algorithm) to obtain the least square estimates of the parameters. Analysis of variance was used to compare JB feeding damage between buds and flowers of different stages of maturation, and for the data in Fig. 6.5A. Pod and seed data from protected or beetle-damaged umbels (Fig. 6.5B) had unequal variances so were analyzed by the nonparametric Wilcoxon rank sum test. All data analyses were performed using Statistix 10 (Analytical Software 2013).

Results

Extent of infestation

A census to gauge extent of JB florivory on *A. syriaca* at three periurban field sites in central Kentucky revealed beetle aggregations and feeding damage to umbels on 98% (98/100), 90% (180/200), and 93% (185/200) of 500 total plants. Extent of floral damage was assessed by bagging 18 umbels with naturally-occurring aggregations in the field, removing and counting the beetles, and then dissecting the umbels and examining individual flowers under a binocular microscope. Aggregation size ranged from 12 to 288

JB per umbel (mean ± SE: 68 ± 16), with an asymptotic relationship between aggregation size and percentage of damaged flowers (Fig. 6.3). Sex ratio within aggregations was male-biased (mean ± SE: 57.1 ± 4.5% males; range: 41.2–77.8%, n = 8). Females were mostly feeding, whereas males often were mounted on females or other males and not feeding.

Stage of bloom and floral parts preferred.

To clarify how flower bud development affects susceptibility to JB feeding, we collected similar-sized umbels in different stages of bloom (closed green bud, pink bud, or with open flowers; see Figure 6.6), confined them individually with five female JB per umbel, and evaluated numbers of buds or flowers that were damaged. After 24 h, the JB had damaged 1.7 ± 1.1, 11.6 ± 9.3, and 45.1 ± 8.0% of the individual buds or flowers on umbels of those developmental stages, respectively ($F_{2,9} = 11.7$; $P < 0.005$).

Milkweeds are remarkable in their floral complexity and means by which pollination is accomplished (Wyatt and Broyles 1994). Nectar is secreted within the five stigmatic chambers formed by stiffened, wing-like elaborations of the adjacent anthers, and stored within saccate extensions of staminal tissue, the hoods, which together form the corona. Each pair of adjacent anther wings forms a slit that allows access to the stigmatic chamber. Two sac-like pollinia (masses of pollen) are located on either side of the stigmatic chamber and joined together at the top of the stigmatic slit. When a nectar-seeking insect visits a donor flower, a leg may become caught in a stigmatic slit, dislodging the paired pollinia that become stuck to the pollinator's appendage or body hairs. When the insect visits another plant of the same milkweed species, a pollinium may be inadvertently inserted into the stigmatic chamber of a recipient flower. Successful pollination results in enlargement of one of the carpels, producing a fruit (pod) containing numerous seeds.

Effects of JB florivory on fruit and seed set.

Field-realistic densities of JB (0, 15, or 50 per umbel) were caged in mesh bags (Figure 6.7) on undamaged umbels of common milkweed in natural stands (eight replicates per density on separate plants) and allowed to feed for 24 h, after which the JBs were removed and the bags were replaced to prevent further florivory and left until formation of pods (fruits). Compared to the controls, just one days' feeding by 15 or 50 JB reduced initial pod set by 67 and 90%, respectively (Fig. 6.5a).

The trial was repeated, except this time we bagged umbels with or without natural JB aggregations (mean: 66.7 ± 9.9 per aggregation; range: 13–147) on separate plants (n = 15 per treatment) in the field, left the bags in place for 24 h, removed the JB, and replaced the bags to shield them from further damage as before. Ten of the 14 surviving shoots upon which the umbels were protected from JB produced mature pods that collectively yielded 5658 total seeds (means: 2.3 ± 0.26 pods per umbel, 246 ± 14 seeds per pod). The fifteen umbels that had been fed upon collectively produced only a single fruit that yielded 223 seeds, representing 96.5% reduction in seed set following JB florivory (Fig. 6.5b).

Discussion

Why do JB aggregate and feed on *A. syriaca* umbels? The polyphagous, day-flying beetles have high energetic requirements (Oertli and Oertli 1990) and they will exploit sugar-rich foods including nectar and floral tissues (Potter and Held 2002; Held and Potter 2004; Hammons et al. 2011). They are attracted to floral odors and aggregate in response to feeding-induced volatiles from damaged plant tissues (Held and Potter 2004). Individual milkweed flowers are long-lived (about 5 d for *A. syriaca*) and produce copious amounts of high-sucrose nectar (Wyatt and Broyles 1994; Willson and Bertin 1979). Milkweed pollen germinates in nectar secreted within the stigmatic chamber (Willson and Bertin 1979). *Popillia japonica* chew into the stigmatic hoods of individual flowers to rob the nectar and feed on the ovaries, destroying the flowers before or after pollination and preventing formation of fruit and seeds. The beetles sometimes also feed secondarily on milkweed leaves distal to vein cuts made by specialist milkweed herbivores (Dussourd and Eisner 1987), but the extent of that injury is unlikely to affect plant fitness.

JB florivory on *A. syriaca* is not restricted central Kentucky where the beetle has been abundant for at least 40 years. Similar damage is occurring in other long-infested eastern states, in the US Midwest where the beetle is more recently established, and in the Great Plains at the invasion front (Fig. 6.8). JB populations fluctuate from year to year but because of their affinity for nectar-feeding on *A. syriaca*, they are likely to aggregate on milkweed umbels even in "down" years. Endemic generalist predators, introduced parasitoids, and endemic and introduced pathogens collectively help to suppress JB populations but historically they have not been effective enough to prevent this highly invasive beetles' range expansion, establishment, and severe damage to favored host plants in North America (Potter and Held 2002).

The eastern monarch population faces threats at different locations and times during its multi-generational migration between overwintering sites in the forests of central Mexico and summer breeding grounds in the US and Canada (Stenoien et al. 2016; Thogmartin et al. 2017b; Malcom 2018; Inamine et al. 2016). The recent population decline has been predominantly attributed to loss of overwintering habitat (Brower et al. 2012) and shortage of larval host plants and nectar resources in the key breeding grounds of the US Midwest where increased use of herbicides to kill weeds in genetically-altered, glyphosate-tolerant crops has coincided with a dramatic reduction in milkweed abundance (Pleasants and Oberhauser 2013; Flockhart et al. 2015; Stenoien et al. 2016; Zaya et al. 2017; Pleasants 2017). Demographic analyses suggest that conserving and planting milkweed to restore the carrying capacity of the breeding grounds is important for stabilizing the monarch population (Flockhart et al. 2015; Pleasants 2017; Oberhauser et al. 2017).

In 2015, The White House announced a National Strategy to promote the health of pollinators that included restoring by 2020 sufficient habitat in the United States to support an eastern migratory monarch population of 225 million butterflies occupying 6 ha of overwintering habitat in Mexico (Pollinator Health Task Force 2015). Mexico and Canada subsequently adopted that goal as part of a long-term cooperative agenda to conserve the monarch and its unique migratory phenomenon (White House, North American climate, clean energy, and environment partnership action plan 2016). Planting of milkweed on public and private lands has emerged as a central conservation strategy

(Thogmartin et al. 2017a; Monarch Joint Venture 2018; U.S. Fish and Wildlife Service 2018).

Asclepias syriaca, which is the main larval host plant for monarchs in their summer breeding range in North America accounting for 92% of the butterflies that overwinter in Mexico (Malcolm et al. 1989, 1993; Thogmartin et al. 2017a), has been the focus of nearly all initiatives for restoring and enhancing monarch breeding habitat (Thogmartin et al. 2017a, 2017c; Pleasants 2017). The major vectors of *A. syriaca* pollinia are Hymenoptera and Lepidoptera, particularly large bees and moths (Flockhart et al. 2017; Willson and Mertin 1979; MacIvor et al. 2017), and those floral "generalist" pollinators effect extensive gene flow within and between populations, boosted by wind dispersal of comose seeds (Wyatt and Broyles 1994). Adult JB activity extends from early June to late August (Potter and Held 2002; Fleming 1972) coinciding with the entire reproductive window of *A. syriaca*. Although the JB is unlikely to reduce survival of individual plants, which can clonally reproduce via rhizomes (Wyatt and Broyles 1994), its florivory will limit pollination and outcrossing, and decrease milkweed's capacity to colonize new areas via seeds.

Conclusion and Implications

The effects of JB florivory on fruit and seed set of milkweed have not been considered in existing estimates (Thogmartin et al. 2017a, 2017b; Pleasants 2017) for how much milkweed must be restored to support the aforementioned conservation goals. Given the JB's outbreak status in the US Midwest and its continuing expansion in the main monarch flyways (Center for Environmental and Research Information Systems

2018), this invasive pest is likely to limit outcrossing and reproduction of wild milkweeds, as well as those planted for monarch habitat restoration. The beetle may also impact the milkweed seed industry that is concentrated in the central Midwest and currently provides most of the seed used for monarch habitat restoration, as well as reproduction of other milkweed species, including a number that are formally designated as threatened or endangered (Borders and Lee-Mäder 2015) at state or federal levels.

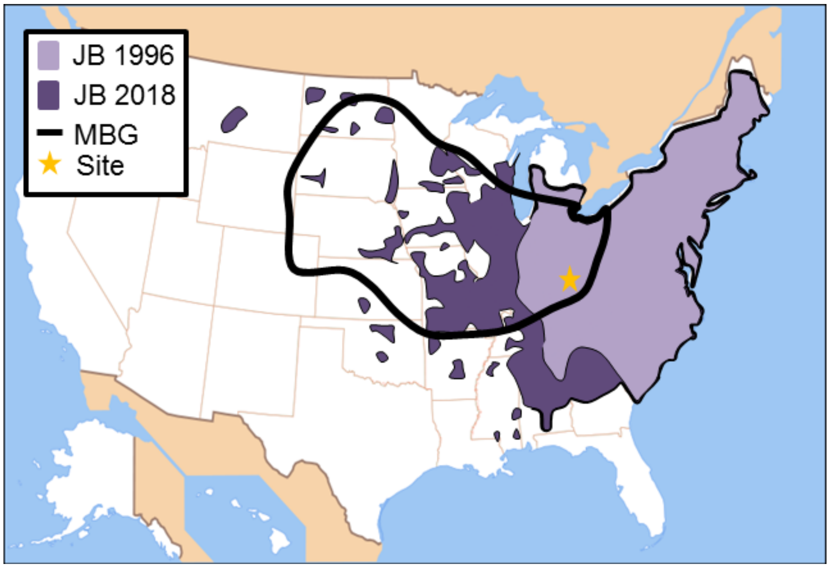

Figure 6.1 Japanese beetle [JB] incursion into the monarch butterfly breeding grounds [MBG] of the US Midwest. JB distributions are based on USDA APHIS Cooperative Agricultural Pest Survey maps (Center for Environmental and Research Information Systems, 2018). Light purple denotes areas occupied by JB in 1996; dark purple denotes additional areas where JB had become established by 2018. Black line encloses the geographic region of the United States that is estimated, based on stable isotope analysis and geospatial modeling, to have produced the highest proportion of monarchs overwintering in Mexico over a 38-year period from 1976–2014 (Flockhart et al. 2017). Star represents the location where the research described herein was conducted.

Figure 6.2 Japanese beetle [JB] feeding on common milkweed, *Asclepias syriaca*. (A) Aggregation of 288 JB on milkweed umbel (inflorescence). Infestations and florivory were widely observed in 2016–2017 and occurred on >90% of surveyed plants. (B) JB biting into coronal hoods of individual flowers.

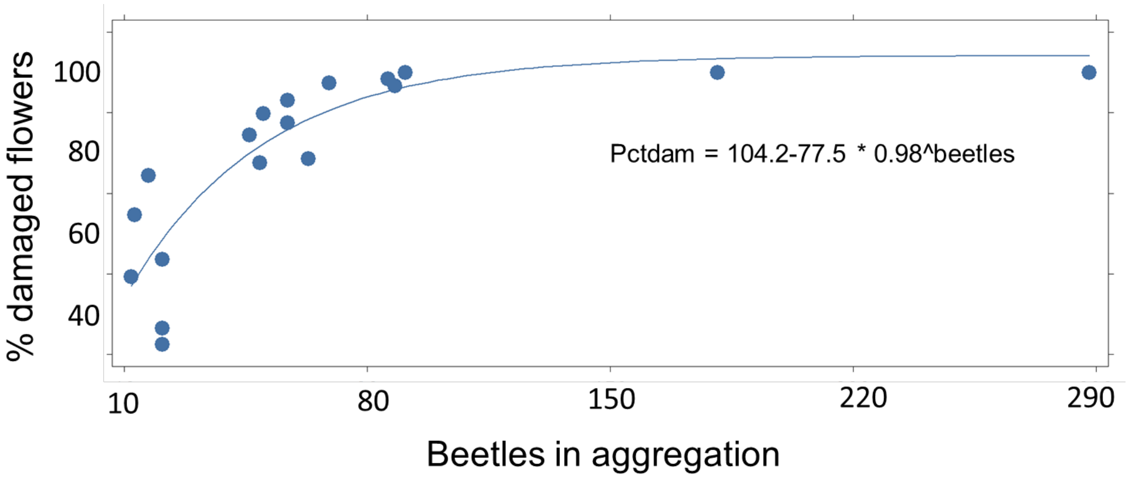

Figure 6.3 Non-linear regression fitted curve showing asymptotic relationship between number of Japanese beetles in natural aggregations on *A. syriaca* umbels and percentage of flowers already damaged**.** At the time of collection, aggregations of 40 or more JB had destroyed 75–100% of the individual flowers.

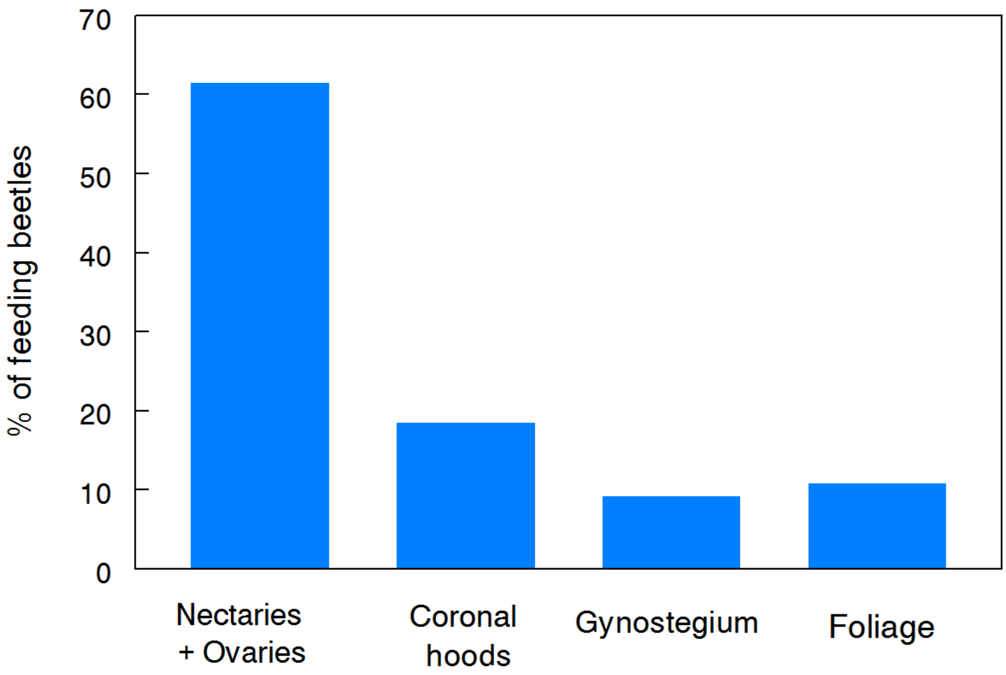

Figure 6.4 Frequency distribution of Japanese beetles [JB] feeding on floral organs or foliage of *A. syriaca* in choice tests. Flowers were dissected into component parts: nectaries+ ovaries (on pedicel), coronal hoods, gynostegium (stigmatic chambers + pollinaria) and offered to individual females (n = 80) in four-way choice tests that included a 1 cm^2 piece of leaf tissue. Food choice of JB that fed (n = 65) differed significantly from the null hypothesis of no preference ($\chi^2 = 47.6$, df = 3, $P < 0.001$).

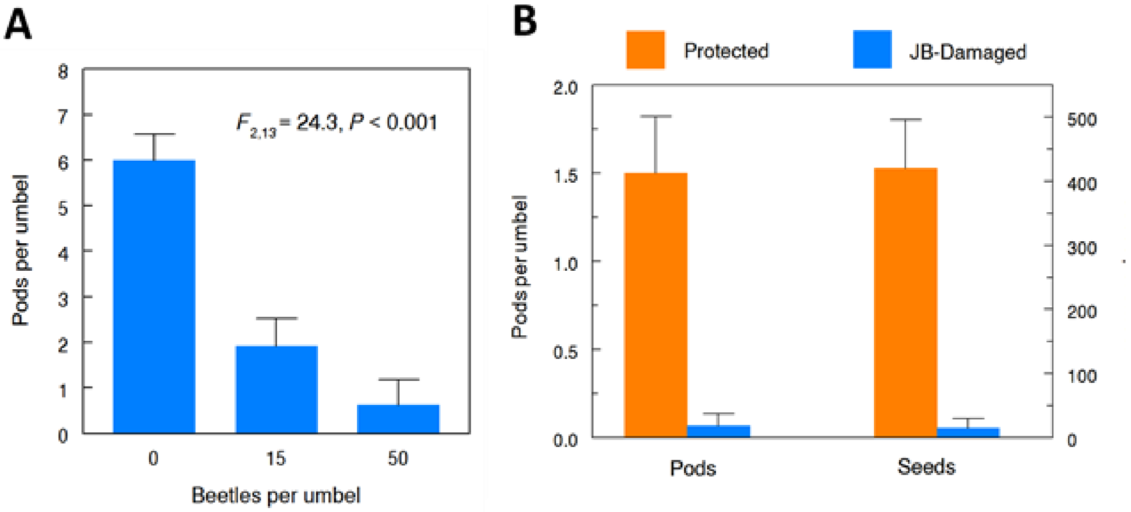

Figure 6.5 Japanese beetle [JB] feeding on umbels reduces milkweed fruiting and seed set. (A) Field-realistic densities of JB caged on intact umbels for 24 h reduced early fruit set. (B) Damage from natural JB aggregations greatly reduced numbers of mature pods and seeds (Wilcoxon rank sum test, $P < 0.001$). Bars represent means + standard error.

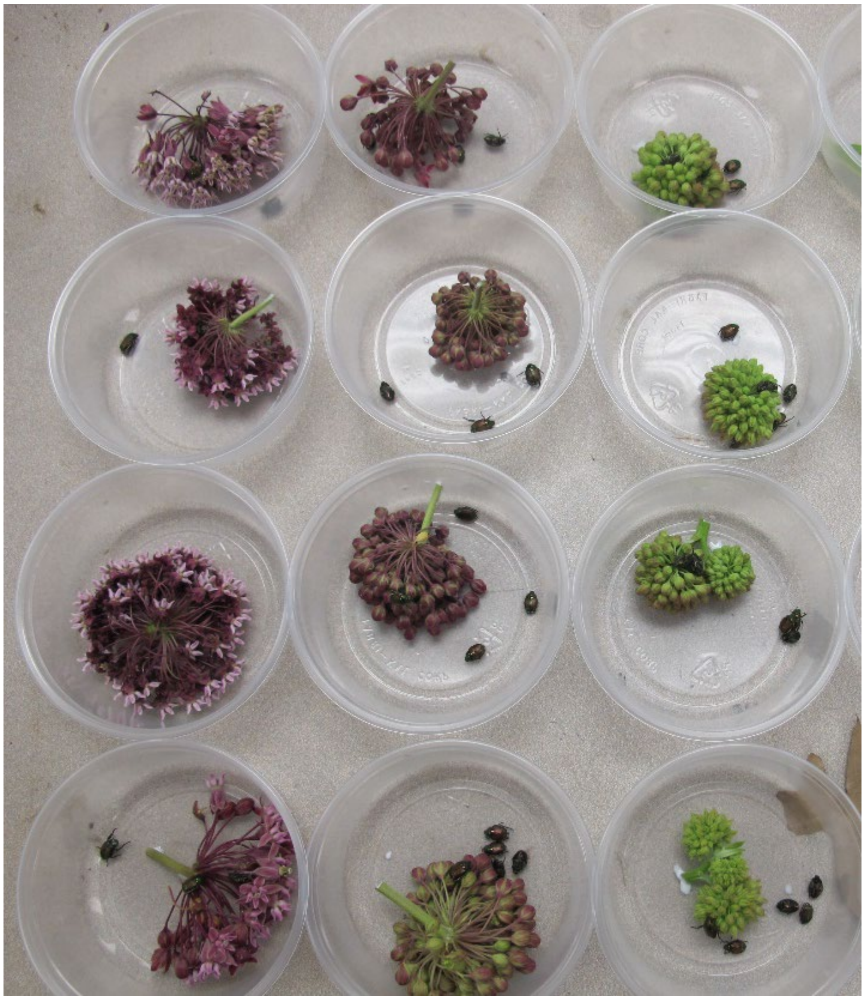

Figure 6.6 To clarify how milkweed flower bud development affects susceptibility to feeding by *P. japonica*, field-collected *Asclepias syriaca* umbels with (left to right) open flowers, pink buds, or closed green buds were confined with five female beetles for 24 h, after which number and percentage of damaged buds or flowers was evaluated.

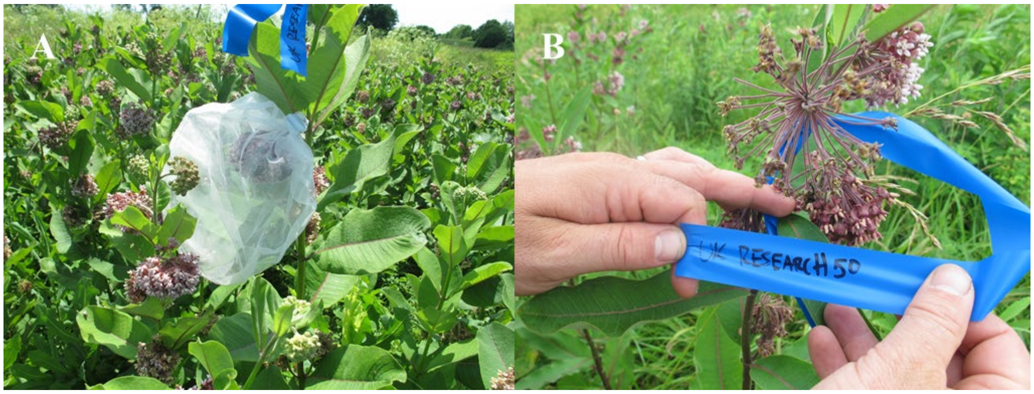

Figure 6.7 (A) Mesh cage enclosing *P. japonica* aggregation on *A. syriaca* umbel. (B) Damage to umbel after 24 h feeding by aggregation of 50 beetles.

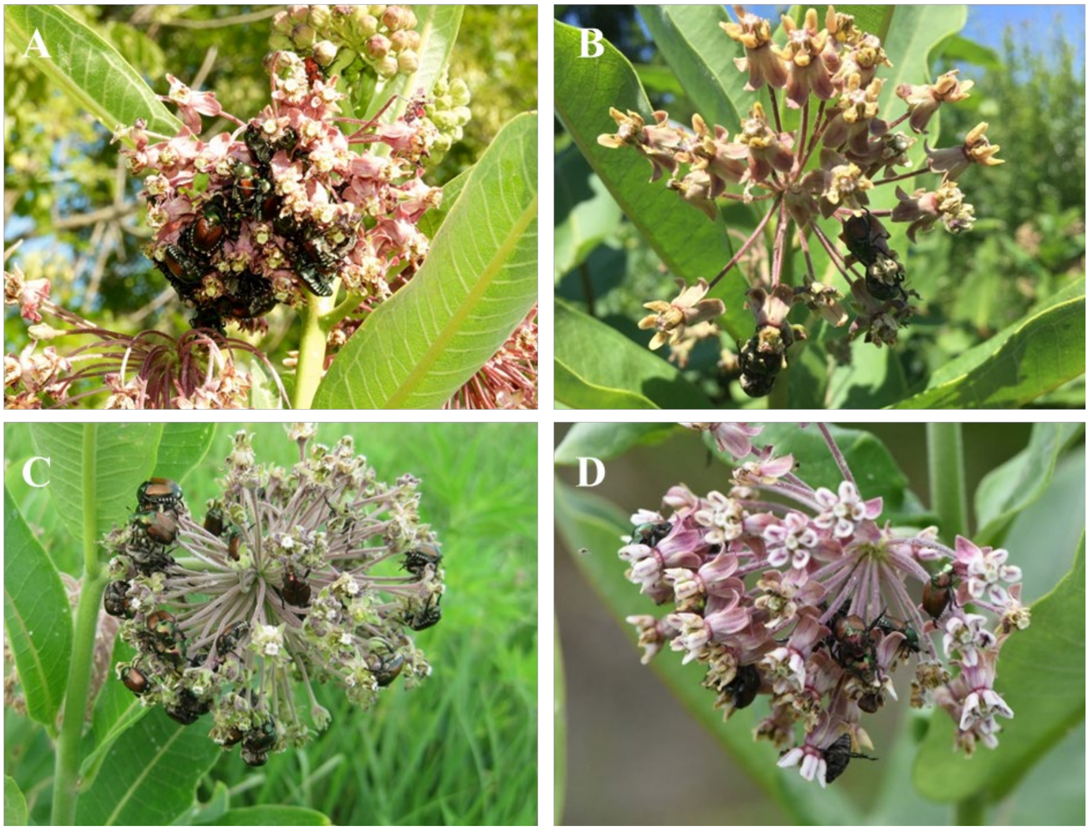

Figure 6.8 (A) *P. japonica* feeding on umbel of *A. syriaca* in Ohio where the beetles have been long established (Photo: C.E. Young). (B) Small aggregation of *P. japonica* feeding on milkweed umbel in Minnesota, with damage from nectar-robbing (coronal hoods have been removed to access ovaries and nectaries) (Photo: B. Thilmony). (C) and (D) Aggregation of *P. japonica* feeding on milkweed umbel in Iowa and Nebraska, respectively, near the invasion front (Photos: L. Iles and T. Weissling).

CHAPTER 7

Summary and Implications

Summary

Planting milkweeds on public and private lands has emerged as a central conservation strategy for restoring declining North American migratory populations of the monarch butterfly. Nearly all actionable science on this issue has focused on restoring common milkweed (*A. syriaca* L.) in rural land types. The overarching goal of my dissertation research was to investigate ways to enhance the conservation value of small urban gardens to support both monarch butterflies and bees. I also studied the impacts of two invasive species, *Popillia japonica* (Japanese beetle) and *Polistes dominula* (European paper wasp), in the context of milkweed restoration and monarch butterfly conservation.

Eight milkweed species varying in height, form, and leaf shape were grown in a common-garden experiment at a public arboretum. I measured milkweed growth, tillering, and bloom periods, conducted bi-weekly counts of eggs and larvae to assess colonization by wild monarchs, and evaluated their suitability for growth of monarch larvae. I also quantified bee visitation and compared the bee assemblages associated with six of the eight species, augmented with additional collections from other sites. Monarchs rapidly colonized the gardens, but did not equally use all of the milkweed species. More eggs and larvae were found on taller, broad-leaved milkweeds, but there was relatively little difference in larval performance, suggesting ovipositional preference for more apparent plants. *Asclepias tuberosa* and *A. fascicularis* attracted the greatest number of bees, whereas bee genus diversity was greatest on *A. verticillata, A. fascicularis*, and *A.*

tuberosa. Milkweeds that do not spread extensively by tillering may be best suited for managed gardens. Combining milkweeds that are preferred by ovipositing monarchs with ones that are particularly attractive to bees may enhance conservation value of small urban gardens.

Ecological theory predicts that specialist insect herbivores are more likely to locate and colonize host plants growing in relatively sparse or pure stands compared to host plants growing amongst diverse non-host vegetation. I tested the hypothesis that increasing the apparency and accessibility of milkweed host plants in small polyculture gardens would boost colonization by the monarch butterfly, an iconic native species of conservation concern. I established replicated gardens containing the identical mix of milkweeds, flowering nectar sources, and non-host ornamental grasses but arranged in three different spatial configurations that were monitored for monarch colonization over two successive growing seasons. Monarch eggs and larvae were 2.5–4 times more abundant in gardens having milkweeds evenly spaced in a 1 m corridor around the perimeter, surrounding the nectar plants and grasses, than in gardens in which milkweeds were surrounded by or intermixed with the other plants. Predator populations were similar in all garden designs. In a corollary open-field experiment, female monarchs laid significantly more eggs on milkweed plants that were fully accessible than on milkweeds surrounded by non-host grasses of equal height. In addition, I monitored monarch usage of 22 citizen-planted gardens containing milkweed and nectar plants in relation to their botanical composition, layout, and surrounding hardscape. Multivariate analysis explained 71% of the variation, with significantly more eggs and larvae found in gardens having milkweeds spatially isolated as opposed to closely intermixed with non-host plants, and in gardens having 100 m north/south access unimpeded by structures.

The decline of native biodiversity in North America has ignited interest in conservation gardening using native plants to support insectivorous birds, pollinators, and other desirable wildlife. Concurrently, the creation of cultivated varieties of native plants, often referred to as ‘nativars’, that have ornamental qualities such as color, stature, bloom display, and disease resistance, is a growing trend in the nursery trade. Native plant cultivars, nevertheless, are not without controversy, and consumers want to know "do they serve the same ecological functions and provide the same benefits to bees and butterflies as wild-type native plants?" I used the high-profile milkweed and monarch system to test the hypothesis that nativars can serve similar ecological functions as wild-type milkweeds in garden settings. In a common garden field experiment I found no difference in colonization over two growing seasons between wild-type *A. incarnata* and *A. tuberosa* and their cultivars. Some cultivars had higher levels of trichomes, latex, or cardenolide concentrations compared to the wild-types, but those differences did not significantly influence larval growth and development. Bee and butterfly communities were similar amongst wild-type milkweeds and their cultivars with exception to ‘Blonde Bomshell’, which had lower bee diversity comprised mainly of bees in the genus *Lasioglossum*. I also compared the non-native tropical milkweed and its cultivars for the larval growth and development and defensive characteristic expression and found no overall differences that influenced monarch growth and development.

Polistes dominula, the European paper wasp (EPW) is an invasive predator that nests in anthropogenic habitats on structures. Because of their abundance in urban areas they may exert strong predation pressure on monarch larvae in garden settings. EPW was the most abundant paper wasp I observed foraging in urban pollinator gardens in central Kentucky. I observed and documented 120 encounters between EPW and monarch larvae on milkweed plants in gardens. Second to fourth instars are at high risk of predation, whereas most fifth instars are able to escape EPW attacks by thrashing or dropping off the plant. The wasps usually bite and carry off second instars whole, whereas third and fourth instar kills are first gutted, then processed and carried away piecemeal. Sentinel larvae left in urban gardens for 8 h experienced 50% predation by *Poilistes* wasps, whereas rural sites only experienced 10%. A census of butterfly boxes in urban pollinator gardens found they are used by EPW as nesting habitat. Putting such boxes in butterfly gardens is likely to be counterproductive. My findings suggest that EPW is an under-recognized mortality factor that can turn urban gardens into ecological traps for monarch larvae and potentially diminish the urban sector's contributions to monarch habitat restoration.

Habitat restoration, including adding millions of host plants to compensate for loss of milkweed in US cropland, is a key part of the international conservation strategy to return the monarch butterfly to sustainable status. I showed that that *P. japonica*, a polyphagous, invasive scarab, aggregates and feeds on flowers of *A. syriaca*, the monarch's most important larval food plant, reducing fruiting and seed set by >90% and extensively damaging milkweed umbels in the field. The beetle's ongoing incursion into the monarch's key breeding grounds in the US Midwest is likely to limit pollination and outcrossing of wild and planted milkweeds, reducing their capacity to colonize new areas via seeds. *Popillia japonica* represents a previously undocumented threat to milkweeds that should be considered in models for monarch habitat restoration.

Implications

The monarch is celebrated in festivals across all of North America. It has the power to inform our scientific literacy, shape our environmental policies, and inspire our horticultural industries. It has been a pleasure to see the influence of this insect on gardeners, naturalists, and the general public nationwide. That being said, conservation of a butterfly that travels thousands of miles in a spectacular annual migration is a complicated business. Many factors influence monarch population success including loss of habitat, “acts of God”, changing climate, pesticides, milkweed scarcity, reduction of overwintering sites, invasive species, predation, windshield induced mortality, disease, parasites, and many other factors. As researchers we are tasked to ask questions that give us a glimpse into this infinitely complicated issue, our only metric for success being the annual overwintering butterfly count of which we cannot directly accredit any conservation effort or the ‘all hands on deck’ efforts (Thogmartin et al. 2017a). We are left to work off the assumption that more milkweed and nectar plants means more monarchs.

This in mind, I have shaped my research to answer questions that lead to actionable science by conservationists, citizen scientists, and backyard ecologists. Regardless of the impact that such research may have on monarch populations, the educational and therapeutic value of gardening for monarchs and other pollinators is undeniable. My research suggests guidelines for garden composition, design, and placement that can help make the urban sector's contributions to monarch habitat restoration more rewarding for participants, and of greater potential value to monarch recovery. It also highlights interactions with two invasive pests that have the potential to hinder monarch butterfly conservation efforts.

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