

Oviposition preference and larval performance of monarch butterflies (*Danaus plexippus*) on two invasive swallow-wort species

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Abstract

The potential of two invasive herbaceous vines *Vincetoxicum nigrum* (L.) Moench and *Vincetoxicum rossicum* (Kleopow) Barbar. (Asclepiadaceae) to reduce monarch butterfly (*Danaus plexippus* L.) (Lepidoptera: Nymphalidae, Danainae) populations was investigated by evaluating oviposition selection in adult monarch butterflies and larval feeding preference in choice tests comparing the native host plant of monarch butterflies, *Asclepias syriaca* L. (Asclepiadaceae) and the two non-indigenous *Vincetoxicum* species. In both choice and no-choice tests, no eggs were oviposited on either of the two *Vincetoxicum* species whereas over 66 eggs per female were oviposited on *A. syriaca* plants. All first instar larvae allowed to feed on *A. syriaca* for 48 h survived while a significantly lower proportion survived on *V. rossicum* (44%) and *V. nigrum* (14%). Mean weight of larvae that did survive on the *Vincetoxicum* species was significantly lower than the mean weight of larvae that fed on *A. syriaca*. The mean weight of surviving larvae, however, did not differ between the two *Vincetoxicum* species. The mean proportion of leaves consumed by larvae feeding on *A. syriaca* was significantly greater than the mean proportion of leaves consumed by larvae feeding on either *Vincetoxicum* species. Findings from this research indicate that *V. rossicum* and *V. nigrum* are not viable hosts of monarch butterflies and are likely to pose little direct threat to their populations as oviposition sinks. The ability of these highly aggressive plants, however, to out-compete and displace the native host of monarchs, *A. syriaca*, may pose a more serious threat. The potential of monarch populations to adapt to the two *Vincetoxicum* species as host plants over the long-term is discussed.

Introduction

The invasion of exotic plant species is recognized as one of the major potential dangers facing many ecosystems (Mack et al., 2000; Pimentel et al., 2000; Wilcove et al., 2000). Invasive plants have been shown to have substantial influence on both plant (Higgins et al., 1999) and animal (Pimentel, 2002) communities. These changes can occur either through direct competition between native and introduced plants or indirectly through changes in the local microclimate (Dukes & Mooney, 2002).

Two invasive plant species of particular concern throughout a large portion of the north-eastern United

States are *Vincetoxicum nigrum* (L.) Moench (syn. = *Cynanchum nigrum*) [black swallow-wort or black dog-strangler vine] and *Vincetoxicum rossicum* (Kleopow) Barbar. (syn. = *Cynanchum rossicum*) [pale swallow-wort or dog-strangler vine] (Asclepiadaceae). These two European species are invasive perennial herbaceous vines of natural and seminatural areas in the north-eastern United States and adjacent south-eastern Canada. *Vincetoxicum nigrum*, a native of western Mediterranean regions, is more widely distributed than *V. rossicum* in North America, and is found from southern Ontario to southern Pennsylvania and from the north-eastern Atlantic coast to Missouri and Kansas to the west (Pringle, 1973; Gleason & Cronquist, 1991; Sheeley & Raynal, 1996). *Vincetoxicum rossicum* is native to the Ukraine and south-western European Russia and is currently most abundant in the lower Great Lakes Basin, but is also found from as far east as Maine and as far

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west as Iowa. The earliest North American herbarium specimen of *V. nigrum* was collected in Ipswich, Essex County, Massachusetts, USA in 1854 and the first collection of *V. rossicum* at Toronto Junction, Ontario, Canada in 1889 (Moore, 1959). The first collections of *V. rossicum* in the north-eastern USA were near Rochester, NY and on Long Island, NY in 1897 (Sheeley, 1992). It is not clear how these species were first introduced into North America, but label information from herbaria specimen suggest that these plants may have been initially grown in botanical gardens and spread thereafter (A. DiTommaso, unpubl.).

The above-ground morphology of the two species is nearly identical except for the flower color from which their names are derived. Below ground, *V. nigrum* is a clonal species with a deep rhizome system (Lumer & Yost, 1995) while no rhizome connections between culms of *V. rossicum* have been found (Sheeley, 1992; Christensen, 1998; Lawlor, 2000). Both *Vincetoxicum* species are members of the Asclepiadaceae (milkweed) family and are associated with disturbed and waste areas such as quarries and transportation corridors. Once established, however, these aggressive species readily move into nearby, less disturbed forest understories, Christmas tree plantations, or reduced tillage agricultural fields, often forming monospecific stands and displacing resident vegetation including the native perennial herb *Asclepias syriaca* L. (common milkweed) (Christensen, 1998; Casagrande & Dacey, 2001; A. DiTommaso, pers. obs.) as well as plant and animal species that are considered threatened or endangered (Bonanno, 1999; Lawlor, 2000).

Recently, there has been increasing concern over the deleterious effects of these highly invasive and aggressive species, not only because of their potential to displace and out-compete threatened or endangered native plant and animal species in natural and seminatural habitats (Bonanno, 1999), but also because of their potential to attract ovipositing monarch butterflies (*Danaus plexippus* L.) (Lepidoptera: Nymphalidae, Danainae) and thus reduce their populations in regions of North America where the two invasive weeds are found (Haribal & Renwick, 1998; Casagrande & Dacey, 2001; Mattila & Otis, 2003).

Although several studies have compared these two invasive species to their native host, *A. syriaca*, no study to date has examined oviposition selection and larval feeding preference of monarch butterflies in the presence of both species. As the geographic distribution of each of these invasive plants continues to expand, it is likely that their ranges will overlap and will result in the three species (including *A. syriaca*), at least initially, occupying many of the same habitats. Thus, it is important to assess the relative attraction of monarch butterflies to these three plant species when all co-occur in the same habitat. The

objectives of this study were: (1) to evaluate oviposition selection in adult monarch butterflies, and (2) to determine larval feeding preference in choice tests comparing the native host plant of monarchs, *A. syriaca*, and the two *Vincetoxicum* species.

Materials and methods

Plant collection

Asclepias syriaca and *V. rossicum* plants were collected in June 2002 from an old-field near Ithaca, NY, USA, and from a field-edge population near Aurora, NY, USA, respectively. *Vincetoxicum nigrum* rhizomes were collected from a Rhode Island, USA, population in August 2001. Intact rhizomes of *V. nigrum* were placed in 2 liter plastic pots containing potting soil (1:1 sphagnum peat : vermiculite) and grown under glasshouse conditions for 9 months prior to being placed outdoors in mid-May 2002. Plants were cut back to a height of 10 cm to encourage tillering before placing outdoors. Plants of *A. syriaca* and *V. rossicum* were potted as for *V. nigrum* and also cut back to a height of 15 and 10 cm, respectively, twice, once in mid-July and once in early August 2002. All plants were watered as needed and fertilised weekly with 500 ml of a 1.25 g l⁻¹ 20-20-20 (N-P-K) fertiliser solution prior to the start of trials in early September 2002. *Vincetoxicum* plants were, on average, 30 cm tall and had three to four tillers at the start of the oviposition experiments, whereas *A. syriaca* plants were, on average, 25 cm tall and had two to three tillers. All flowers were removed from plants at the beginning of the trials.

Monarch butterfly source

Mated female monarch butterflies were obtained from a captive colony in Ithaca, NY, USA that had been reared on *Asclepias curassavica* L. plants. Females were exposed to males for 3 days beginning 4 days after emergence from pupae.

Adult host preference tests

Oviposition trials were initiated on 3 September 2002 and performed under glasshouse conditions at a constant temperature of 26 ± 4 °C, 60 ± 10% r.h., and L14:D10. A single mated female butterfly was randomly assigned to one of seven screened cages measuring 0.6 × 0.6 × 0.6 m in size and containing a single potted *A. syriaca*, *V. nigrum*, and *V. rossicum* that were approximately the same height and leaf surface area. Care was taken not to have the test plants touch each other in the cages. Sugar water in small sponges was provided as an energy source. Adult butterflies were released into the cages for a period of 48 h, after which they were removed and the number of eggs laid on each of the three plant species was recorded. Following

Table 1 Comparison of adult preference and larval performance of *Danaus plexippus* on *Asclepias syriaca*, *Vincetoxicum rossicum*, and *Vincetoxicum nigrum* in laboratory bioassays

Parameter*	Plant species		
	<i>A. syriaca</i>	<i>V. rossicum</i>	<i>V. nigrum</i>
Oviposition			
Mean no. of eggs per plant	66.57 ± 23.96 a	0.00 ± 0.00 b	0.00 ± 0.00 b
Larval survival			
Mean proportion surviving	1.00 ± 0.00 a	0.44 ± 0.05 b	0.14 ± 0.08 c
Larval weight			
Mean weight per surviving larvae (mg)	2.57 ± 0.18 a	0.52 ± 0.04 b	0.59 ± 0.12 b
Leaf consumption			
Mean proportion leaves consumed	0.51 ± 0.07 a	0.03 ± 0.01 b	0.02 ± 0.01 b

*Values for each parameter denoted with the same letter are not significantly different ($P > 0.05$), $n = 7$, and d.f. = 18 for analysis of all parameters.

this 48-h period, potted plants of *A. syriaca* were removed from the cages, leaving only *Vincetoxicum* species in each cage. The single adult female butterfly was once again allowed to oviposit for an additional 48 h. Following this period, the number of eggs laid on each of the *Vincetoxicum* species was determined. Data from the three-plant experiment were analysed with ANOVA to test for an overall effect of treatment and means for individual plant species were separated with Bonferroni tests at the $P < 0.05$ level of significance (SAS Institute, 2002). No eggs were oviposited in any cage when only the two *Vincetoxicum* species were tested so these data were not analysed.

Larval feeding preference

Larvae used for these experiments were obtained from eggs laid and hatched on *A. syriaca* plants from the trials described previously. All testing was carried out in ventilated Petri dish arenas (15 cm in diameter) using detached leaves of approximately equal size from each of the three test species. Leaves were partially rolled and petioles inserted into a 10-ml test tube containing de-ionized water. Test tubes were sealed with parafilm to prevent water loss and arenas were covered with a fine mesh. Seven replicate leaves of each plant species were used. Ten first instar larvae (< 24 h old) were placed gently with a paintbrush on each leaf and allowed to feed for a period of 48 h. Larvae were drawn randomly from eggs oviposited by multiple females. After 48 h, the number of larvae surviving and the average weight of surviving larvae on each leaf were determined. A visual estimate of the percentage of leaf area consumed by larval feeding was also recorded after 24 and 48 h. Leaf consumption fell into two categories, with leaves either less than 70% consumed or greater than 95% consumed. For leaves more than 90% consumed the proportion consumed was estimated to the nearest 1% for all other leaves, the proportion consumed was estimated to the nearest 10%. This two-tiered system

tends to overestimate variance of less consumed leaves making it less likely that differences would be found and thus more conservative. This efficient method was, however, sufficient to find significant differences between treatments. The proportion of larvae surviving, the final larval weights, and the proportion of leaf area consumed were analysed with ANOVA to test for an overall effect of treatment and means for individual plant species were separated with Bonferroni tests ($P < 0.05$) (SAS, 2002). Proportional data were arc-sine transformed prior to analysis to insure homoscedasticity.

Results

In the three-species oviposition experiment, no eggs were oviposited on either *Vincetoxicum* species while the mean number of eggs on *A. syriaca* was over 66 per plant (Table 1). No eggs were oviposited on either *Vincetoxicum* species in the subsequent experiment when *A. syriaca* was removed.

All larvae feeding on *A. syriaca* survived while a significantly lower proportion survived on *V. rossicum* and *V. nigrum* (Table 1). A significantly higher proportion of larvae survived on *V. rossicum* compared with *V. nigrum* (Table 1). The mean weight of larvae that did survive on the *Vincetoxicum* species was significantly lower than the mean weight of larvae that fed on *A. syriaca* (Table 1). The mean weight of surviving larvae from the two *Vincetoxicum* species was not significantly different. The mean proportion of leaves consumed by larvae feeding on *A. syriaca* leaves was significantly greater than the mean proportion of leaves consumed by larvae feeding on either *Vincetoxicum* species (Table 1).

Discussion

The two invasive *Vincetoxicum* species we studied could potentially have either a positive or a negative impact on

monarch butterfly populations. Assuming monarchs are host limited to some degree, if invasion by *Vincetoxicum* species leads to an increase in the availability of viable hosts, then the impact on monarch populations will likely be positive. Based on our results this seems very unlikely. Our data show that less than half of the larvae placed on either *Vincetoxicum* host survive for 48 h. This corroborates other previous studies that evaluated the species individually and found no larvae surviving to maturity (Haribal & Renwick, 1998; Casagrande & Dacey, 2001; Mattila & Otis, 2003). Taken together these results indicate that *V. rossicum* and *V. nigrum* are not viable hosts. This contention is supported by the average weight of larvae feeding on the two *Vincetoxicum* species, which was less than one-quarter of the weight of larvae that fed on *A. syriaca* after 48 h. The low levels of leaf consumption on *Vincetoxicum* plants demonstrate that the impaired performance results from non-preference for the invasive plants rather than inability to assimilate material consumed. If death occurred later in development or at higher weights then monarchs might quickly adapt to these new plant species. All the available data suggest, however, that adaptation to these hosts is unlikely in the short-term and that these plants may be a high mortality 'sink' for a portion of the monarch population.

The impact of the *Vincetoxicum* species as a sink for monarch populations will depend on the proportion of overall oviposition that occurs on them as opposed to viable hosts. Fortunately, our results and data from previous studies point to a very low level of oviposition on these two species. Although other cage studies found higher levels of oviposition on both *V. rossicum* (0.5%; Mattila & Otis, 2003) and *V. nigrum* (21.7%; Casagrande & Dacey, 2001), no intact monarch eggs were found on *V. nigrum* where it was in proximity to *A. syriaca* in the field. Thus, it would appear that these two *Vincetoxicum* species do not pose a serious threat to the monarch butterfly as a high mortality sink. Nonetheless, no fewer than 54 rare species of plants, insects, birds, and land snails are threatened by *V. rossicum* invasion of the globally rare alvar (i.e., shallow limestone barrens) habitats in New York State's Great Lakes Basin (Bonanno, 1999). Recent work by Greipsson & DiTommaso (2002) has also revealed an alteration in the composition and activity of the arbuscular mycorrhizal fungal (AMF) soil community following colonization of *V. rossicum* in several contrasting habitats of central New York State, USA. The long-term effect of altered AMF structure and activity in soil on ecosystem structure and function is not yet known.

Although the threat of a monarch butterfly mortality sink seems minimal, these *Vincetoxicum* species could still negatively impact monarch populations by displacing

viable native hosts such as *A. syriaca*. There are many examples of native plants being reduced or even eliminated from portions of their range by invasive species (e.g., Mack, 1981; D'Antonio, 1993; Meekins & McCarthy, 1999; Stohlgren et al., 1999). Determination of the extent of this threat will require data on competition between native and invasive plants in the family Asclepiadaceae and data on the level to which monarch populations are host-plant limited.

While the short-term impact of *V. rossicum* and *V. nigrum* will be determined by ecological processes such as competition between plants, the long-term impact of these species will be determined by evolutionary processes such as adaptation to a new host plant by the monarch butterfly. Other insects endemic to North America have adapted to invasive weeds. For example, the aquatic weevil, *Euhrychiopsis lecontei* Dietz (Coleoptera, Curculionidae), has adapted to exploit the exotic weed, Eurasian watermilfoil, *Myriophyllum spicatum* L. (Sheldon & Creed, 1995). By comparing both species of *Vincetoxicum*, we have determined that survival was significantly higher on *V. rossicum* than *V. nigrum*. In addition, no larvae survived to 48 h in four of the seven replications with *V. nigrum* while more than 28% of the larvae survived in each replication with *V. rossicum*. This may indicate that although no monarch larvae yet tested can complete development on either *Vincetoxicum* species, adaptation may be more rapid on *V. rossicum*. This could have important implications for monarch populations, especially in those areas where *V. rossicum* is most common.

Our results on the interactions of *V. rossicum* and *V. nigrum* with monarch butterflies have short-, medium-, and long-term implications. In the short-term, it is clear that the two *Vincetoxicum* species studied are not presently hosts or sinks for monarch populations. Based on this finding, direct negative impact on monarch populations should not be used as a justification for control of these aggressive invasive plants. Conversely, because these *Vincetoxicum* species are not currently viable hosts for monarchs, controlling them should not negatively impact monarch populations unless control measures (e.g., use of broad-spectrum herbicides) have unintended effects on viable hosts such as *A. syriaca*.

In the medium- and long-term (3–10 years), the level to which these invasive species are displacing native host plant species needs to be monitored. Clearly, if the availability of *A. syriaca* and other viable host plants declines, monarch populations will be negatively impacted. Monarch populations should also be monitored to assess potential adaptation to these new host plants. This may be especially important for *V. rossicum*, because survival rates are significantly greater than for *V. nigrum*.

Vincetoxicum rossicum and *V. nigrum* are established in North America and their range continues to expand (A. DiTommaso, unpubl.). Directed studies on the ecology and invasive potential of these non-native species in their introduced North American range will contribute to the development of effective management strategies that will have minimal negative impacts on endemic plant and animal species.

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