Photoperiodic Response of Abrostola asclepiadis (Lepidoptera: Noctuidae), a Candidate Biological Control Agent for Swallow-worts (Vincetoxicum, Apocynaceae)

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Understanding the potential efficacy of a candidate weed biological control agent has become more prominent in recent years, especially pre-release studies of an agent’s impact on plant performance (e.g., Grevstad et al. 2013, Reddy and Mehelis 2015). Such assessments have been proposed for prioritizing agents for further study or release, in order to enhance the success rate of weed biological control programs (Balciunas and Coombs 2004, McClay and Balciunas 2005).

The seasonal duration and amount of damage that an agent might inflict on a targeted invasive plant will depend in part on the number of agent generations (voltinism) produced in the area of release. The induction of diapause in an insect population is a key component determining the number of generations and seasonality of insect activity. For many insects of temperate zones, photoperiod is a primary cue for diapause induction (Tauber et al. 1986).

Black swallow-wort [Vincetoxicum nigrum (L.) Moench = Cynanchum louiseae Kartesz and Gandhi] and pale swallow-wort [V. rossicum (Kleopow) Barbar. = Cynanchum rossicum (Kleopow) Borhidi] (Apocynaceae-subfamily Asclepiadoideae) are European twining vines introduced into northeastern North America. One candidate agent is the defoliator Abrostola asclepiadis (Denis and Schiffermüller) (Lepidoptera: Noctuidae). The moth reportedly has up to two generations in parts of its native range. We assessed the potential multivoltinism of Russian and French populations of the moth by rearing them under constant and changing photoperiods, ranging from 13:11 to 16:8 hour (L:D). The French population was also reared outdoors under naturally-changing day lengths at a latitude similar to northern New York State. Less than six adult moths emerged, with one exception, for any photoperiod treatment. We expect A. asclepiadis to be univoltine if it were to be released into North America, limiting its potential impact on swallow-worts. It should therefore be given a lower priority for release.

Keywords: Cynanchum rossicum, Cynanchum louiseae, swallow-wort, weed biological control, photoperiod
Erebidæ) has been released in Canada (2013) and more recently in the U.S. (Weed et al. 2011b, Young and Weed 2014, Milbrath and Biazzo 2016).

One candidate biological control agent is the defoliating moth Abrostola asclepiadis (Denis and Schiffermüller) (Lepidoptera: Noctuidae). It is widely distributed in Europe in open field to shaded habitats. In some years and locations it defoliates entire stands of Vincetoxicum hirundinaria Medik., its primary host plant (Foräre 1995, Leimu and Lehtilä 2006). The moth overwinters as a pupa in leaf litter and has one-two generations per year. Two generations have been reported in some areas of central Europe (Foräre 1995) that would have similar latitudes to northern New York and southern Ontario. Abrostola asclepiadis appears to be host specific to Vincetoxicum spp. (Hazlehurst 2011). However, a single defoliation of black or pale swallow-wort has a limited effect on the plants (Weeds et al. 2018, 2019). A bivoltine population of A. asclepiadis that can damage the plants over a greater duration of the season is therefore desired for release into North America. Many noctuid species have facultative diapause, in which the number of generations produced depends in part on the response of populations at a given latitude to environmental cues such as photoperiod (Tauber et al. 1986, Saulich et al. 2017). No diapause-induction studies have been conducted with A. asclepiadis.

Our objective was to determine the potential number of generations of different populations of A. asclepiadis that could occur in the invasive range of swallow-worts. Populations were exposed to photoperiods that would typically experience during the growing season in the northeastern U.S. and southeastern Canada. The results were to be used in concert with separate impact studies and a population matrix model for swallow-worts (Milbrath et al. 2018, 2019) to predict the efficacy of this candidate agent.

**Materials and Methods**

**Insect cultures.** Eggs of Russian A. asclepiadis were collected 29 May–6 June 2013 from Vincetoxicum spp. leaves in the Russian North Caucasus, near Kislovodsk (43°56.400′ N, 42°41.734′ E) and Borgustanskaya (44°02.0436′ N, 42°30.161′ E). Eggs were transported to the Zoological Institute, Russian Academy of Sciences, St. Petersburg for the first diapause experiment (see Initial diapause test). Diapausing pupae were subsequently shipped to the senior author for additional experiments described below. Eggs of French A. asclepiadis were collected 16 June 2014 from V. hirundinaria at Glan-dage, France (44°42.033′ N, 05°37.000′ E). Larvae were reared on V. hirundinaria at the European Biological Control Laboratory (EBCL), Montferrier-sur-Lez, France, under a 10:14 hour (L:D) photoperiod to induce diapause and pupae were shipped to the senior author. Voucher specimens have been deposited with the Cornell University Insect Collection, Department of Entomology, Ithaca, NY (under Lot Number 1263).

Diapausing pupae were overwintered at 5°C and a 10:14 hour (L:D) photoperiod for up to 9 months. Pupae were then transferred to a chamber set at 14:10 hour (L:D) and 25:20°C for adult emergence, mating, and oviposition. Two groups of adult moths were held in 60 × 60 × 60 cm aluminum-mesh cages containing honey-water and potted pale or black swallow-wort plants for oviposition. Leaves with egg masses were collected daily (< 24 hour post-oviposition) to minimize the time at a non-experimental photoperiod.

**Initial diapause test.** Neonate larvae (1st laboratory generation) were placed individually into ventilated 0.25 liter plastic vials and randomly assigned to custom-made programmable chambers set to 20 or 25°C and one of six photoperiod treatments at each temperature (hours, L:D): 13:11, 16:8; 13:11 to 16:8 fast, 16:8 to 13:11 fast, 13:11 to 16:8 slow, or 16:8 to 13:11 slow. A short day of 13 hours represents natural day lengths in the North Caucasus in early-April or early-September, and a long day of 16 hours exceeds the longest summer day length (sunrise to sunset, Fig. 1). For the fast photoperiod change, larvae were transferred to the alternate photoperiod at approximately the third instar or halfway through their larval development (day 11 at 20°C, day 8 at 25°C). For the slow change, day length was increased (or decreased) daily in a stepwise fashion over a 5 day (20°C) or 3 day (25°C) period. Ten larvae were used per treatment combination for each of the two Russian populations for a total of 240 larvae. Larvae were fed excised leaves from Vincetoxicum sp. plants that had also been collected from the North Caucasus region. The larvae were checked daily and leaves were replaced. Upon pupation, pupae were maintained under their same experimental rearing conditions for an additional 40 days, which is over twice the expected time for emergence from non-diapausing pupae (L. Milbrath, unpublished data). Live pupae that had not emerged as adults at the end of this period were considered to be in diapause. Temperature, photoperiod and source population were treated as a single combined factor and tested against the categorical data of diapause (yes or no) using the G-test with simultaneous test procedures (Sokal and Rohlf 1995).
Constant photoperiods. Based on the most likely conditions to promote non-diapause development from the initial diapause test, we used 20°C for further tests with a Russian (Kislovodsk) or French (Glandage) population. The average monthly temperature in Ithaca, NY from June to August ranges from 18.1–20.4°C (NRCC 2018). The experimental design was a one-way treatment structure in a completely randomized design with five constant photoperiods [14:10, 14.5:9.5, 15:9, 15.5:8.5 or 16:8 hours (L:D)]. A short day of 14 hours represents natural day lengths across New York State and north to Ottawa, ON in late-April or mid-August, and a long day of 16 hours exceeds the longest summer day length (Fig. 1). Populations were tested in different years and consisted of larvae of the 2nd laboratory generation.

Groups of egg masses were randomly assigned to one of the five photoperiod treatments in programmable incubators (model I30BLL, Percival Scientific, Inc., Perry, IA 50220). Egg masses were initially placed into 14.5 × 2 cm Petri dishes lined with moist filter paper. At the black-head stage (c. 5 days), groups of c. 80 fertile eggs were transferred to 27 × 19 × 9.5-cm ventilated plastic boxes for larval rearing with five replicates per photoperiod treatment. Boxes contained a false bottom of plastic mesh to allow frass to collect away from developing larvae and plant leaves. Bouquets of greenhouse-grown pale or black swallow-wort stems in water-filled vials were added. Additional bouquets were added as needed until 3rd instar larvae were present, at which time cut stems of field-collected pale swallow-wort were added daily to maintain an excess of food. Black and pale swallow-wort are both suitable hosts for A. asclepiadis (Weed et al. 2011b). Boxes were cleaned of old stems and frass every few days. Prior to eclosion of 4th instars, larvae were randomly culled to 50-55 larvae per box to minimize cannibalism and food shortages.

Upon pupation of all larvae, boxes were cleaned, cocoons were opened and live pupae were placed back into the boxes on a layer of moist vermiculite. Pupae were maintained under their respective experimental rearing conditions and observed for adult emergence over a 50 day period. Remaining pupae were considered alive but in diapause if there was visible movement of the abdomen when gen-
ly squeezed with soft forceps. Dead pupae were dissected. If pharate adults were found, the individual was scored as non-diapausing. All other dead pupae were omitted from analyses. The percentage of diapausing *A. asclepiadis* was calculated as: \% Diapause = \( \frac{\text{Number of live pupae}}{\text{number of emerged adults} + \text{number of live pupae} + \text{number of dead pharate adults}} \) (Miller et al. 2000). Diapausing pupae were subsequently ramped down to 5°C over 4 weeks and overwintered as previously described for use in the next experiment. Percentage diapause data were separately analyzed for the two populations using analysis of variance with a logit transformation (PROC MIXED, SAS Institute 2012). Means were separated using the least-significant difference test (SAS Institute 2012).

**Changing photoperiods.** An experiment was conducted to assess the effect of changing photoperiods on diapause induction for the Russian and French populations (3rd laboratory generation) of *A. asclepiadis*. The experimental design, methods and analysis were identical to those described under **Constant photoperiods** except that two changing and two constant photoperiod treatments were used: 14:10 to 16:8, 16:8 to 14:10, 14:10 and 16:8 hours (L:D). For the changing photoperiod treatments, boxes of larvae were transferred to the alternate photoperiod the day after most larvae in a box molted to 3rd instar.

**Natural photoperiods.** An outdoor observational study was conducted in France at the EBCL (43°41.033' N, 03°52.500' E) over a three-year period using insects from the Glandage population. Day lengths at EBCL are comparable to those in northern New York State (Fig. 1). In 2016 and 2017, eggs and 1st instars of *A. asclepiadis* were collected from the field in June and reared to the 2nd instar in Petri dishes in the laboratory at c. 22°C and under natural day lengths (next to a window). Larvae were then placed on potted *V. hirundinaria* plants, 2–4 larvae per pot, covered with a 50 cm tall mesh bag supported by metal rods. Pots were located outdoors in a semi-shaded location where the larvae experienced naturally-changing day lengths. Pots were watered as needed and observed for adult emergence. Pupae were recovered from all pots in August or September and remained outdoors on a covered and potted plant for additional observations. In 2016, plants were infested with a total of 78 2nd instars on 22 June and 6 July. In 2017, plants were infested with 108 2nd instars on 19–26 June.

Pupae from the 2017 experiment were overwintered outdoors at EBCL, and ten adult moths emerged 7–11 May 2018. Moths were maintained outdoors on a potted and covered *V. hirundinaria* plant and eggs were collected, approximately one month earlier than in the field at Glandage. On 17 May, 107 eggs and 72 1st instar larvae were distributed among 20 potted *V. hirundinaria* plants and pots were covered with a mesh bag. Additional foliage was added to the caged plants to allow larvae to complete their development. Pupation was observed to occur beginning 12 June. Pupae were recovered from all pots on 27 July (c. 45 days after first pupation) and transferred to a glasshouse cage containing potted plants for an additional month of observation under natural day lengths.

**Results**

Varying numbers of non-diapausing moths emerged at 20°C, but not 25°C, for both Russian populations (Table 1). The greatest emergence, and therefore generally

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Photoperiod (hours, L:D)</th>
<th>Kislovodsk</th>
<th>Borgustanskaya</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>13:11</td>
<td>60 (10) ab</td>
<td>100 (10) a</td>
</tr>
<tr>
<td></td>
<td>16:8</td>
<td>100 (10) a</td>
<td>100 (10) a</td>
</tr>
<tr>
<td></td>
<td>13:11 to 16:8 fast</td>
<td>90 (10) ab</td>
<td>90 (10) ab</td>
</tr>
<tr>
<td></td>
<td>16:8 to 13:11 fast</td>
<td>20 (10) b</td>
<td>56 (9) ab</td>
</tr>
<tr>
<td></td>
<td>13:11 to 16:8 slow</td>
<td>100 (9) ab</td>
<td>100 (9) ab</td>
</tr>
<tr>
<td></td>
<td>16:8 to 13:11 slow</td>
<td>100 (10) a</td>
<td>100 (10) a</td>
</tr>
<tr>
<td>25</td>
<td>13:11</td>
<td>100 (10) a</td>
<td>100 (10) a</td>
</tr>
<tr>
<td></td>
<td>16:8</td>
<td>100 (10) a</td>
<td>100 (9) ab</td>
</tr>
<tr>
<td></td>
<td>13:11 to 16:8 fast</td>
<td>100 (9) ab</td>
<td>100 (10) a</td>
</tr>
<tr>
<td></td>
<td>16:8 to 13:11 fast</td>
<td>100 (10) a</td>
<td>100 (10) a</td>
</tr>
<tr>
<td></td>
<td>13:11 to 16:8 slow</td>
<td>100 (9) ab</td>
<td>100 (10) a</td>
</tr>
<tr>
<td></td>
<td>16:8 to 13:11 slow</td>
<td>100 (10) a</td>
<td>100 (10) a</td>
</tr>
</tbody>
</table>

1 Values followed by the same letter are not significantly different (G-test with simultaneous test procedure performed on counts, P > 0.05).
the least percentage diapause, occurred with the Kislovodsk population under the “16:8 to 13:11 fast” treatment. Some appreciable emergence also occurred for this same treatment for the Borgustanskaya population and under a short day (13:11) for the Kislovodsk population (Table 1).

In subsequent tests with daylengths ranging from 14 to 16 hours and much larger numbers of larvae, 0–5 adults of the French or Russian populations emerged from the various constant or changing photoperiod treatments. Percentage diapause was therefore 98–100% across the different tests (Table 2). In outdoor tests in France, one adult moth emerged from 18 surviving pupae in 2016 (94% diapause), no adults emerged from 38 pupae in 2017 (100% diapause), and no adults emerged from 95 pupae in 2018 (100% diapause).

Discussion

Both the Russian (Kislovodsk) and French (Glandage) populations of A. asclepiadis appear to be univoltine under photoperiods they will experience during the spring and summer in the northeastern USA and southeastern Canada (Fig. 1). The fact that the two geographically separated populations in our study showed a similar photoperiodic response suggests that other populations that may be considered for release (e.g., Ukraine, Hazlehurst 2011) would also likely be univoltine, at least under the experimental conditions we provided. In Europe, A. asclepiadis appears to be a primarily univoltine species although bivoltine populations have been reported (Förare 1995, Weed et al. 2011a). Voltinism among other temperate noctuid species in the same subfamily (Plusiinae) as A. asclepiadis is variable (Saulich et al. 2017). Some species are strictly univoltine with an obligate diapause, e.g., Charanyca trigrammica (Hufnagel) (Sokolova 2007). Other species have a facultative diapause and variable numbers of generations depending on the latitude or when offspring are produced in the spring relative to current photoperiods, e.g., the congener A. triplasia (L.) (Saulich et al. 2015).

In this study we did not determine the critical day length for diapause induction in A. asclepiadis, i.e., the day length that results in 50% of the individuals in a population entering diapause. However, a small pilot study in which larvae of the Kislovodsk population were reared at 18:6 hour (L:D) resulted in only 60% diapause (n=10, L. Milbrath, unpublished data). This result and the significant emergence of adult moths for a few treatments in the first diapause test (Table 1) suggest that the Russian populations of A. asclepiadis have a facultative, and not an obligate, diapause (Sokolova 2007). Nevertheless, the experimental conditions observed to promote non-diapause development (>16 hours day length, a rapid 3 hour decrease in day length) are ecologically unrealistic. No such conditions naturally occur in either the area of moth collection or intended introduced range (Fig. 1). The French population may also have a facultative diapause, but the conditions which might promote a second generation are unknown. Rearing the French population outdoors at a typical time (2017 test) or even one month earlier than normal (2018 test) did not result in a (partial) second generation.

### Table 2. Mean (± SD) percentage diapause (total live insects assessed) for two source populations of Abrostola asclepiadis reared under different constant and changing photoperiod regimes, 20ºC.

<table>
<thead>
<tr>
<th>Photoperiod (hours, L:D)</th>
<th>Glandage, France</th>
<th>Kislovodsk, Russia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:10</td>
<td>99.3 ± 1.5 (171) a</td>
<td>100 ± 0 (219)</td>
</tr>
<tr>
<td>14:5:9:5</td>
<td>99.5 ± 1.0 (198) a</td>
<td>100 ± 0 (196)</td>
</tr>
<tr>
<td>15:9</td>
<td>100 ± 0 (189) a</td>
<td>100 ± 0 (197)</td>
</tr>
<tr>
<td>15:5:8:5</td>
<td>99.5 ± 1.1 (199) a</td>
<td>100 ± 0 (181)</td>
</tr>
<tr>
<td>16:8</td>
<td>100 ± 0 (196) a</td>
<td>100 ± 0 (140)</td>
</tr>
<tr>
<td>F4, 20 = 0.50; P = 0.735</td>
<td></td>
<td>No analysis possible</td>
</tr>
<tr>
<td>Changing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:10 to 16:8</td>
<td>99.1 ± 2.0 (196) a</td>
<td>97.8 ± 1.3 (192) b</td>
</tr>
<tr>
<td>16:8 to 14:10</td>
<td>99.5 ± 1.1 (171) a</td>
<td>100 ± 0 (203) a</td>
</tr>
<tr>
<td>14:10 control</td>
<td>100 ± 0 (201) a</td>
<td>100 ± 0 (218) a</td>
</tr>
<tr>
<td>16:8 control</td>
<td>97.6 ± 3.0 (183) a</td>
<td>99.6 ± 1.0 (223) a</td>
</tr>
<tr>
<td>F3, 16 = 1.80; P = 0.188</td>
<td></td>
<td>F3, 16 = 7.57; P = 0.002</td>
</tr>
</tbody>
</table>

1 Within each test and population, individual means followed by the same letter are not significantly different (F-protected LSD test with logit-transformed data, P > 0.05; n=5).
It is unknown if a change in critical day length for diapause induction would occur in *A. asclepiadis* should it be released into North America, allowing for two generations. The critical day length for the biological control agent *Diorhabda carinulata* (Desbrochers) (Coleoptera: Chrysomelidae) was documented to decrease following release, allowing it to extend its range to more southern latitudes (Bean et al. 2012). It is also unknown if sufficient degree-days are available to complete development to the (overwintering) pupal stage should a second generation of *A. asclepiadis* occur in the future. Two generations of *A. asclepiadis* are reported from central Europe and an occasional partial second generation in Sweden is thought to occur, perhaps due to differing seasonal temperatures or critical day lengths (Förare 1995).

The potential impact of *A. asclepiadis* appears to be limited with a univoltine life cycle. Available defoliation impact data of *A. asclepiadis* (Milbrath et al. 2019) combined with swallow-wort population models (Milbrath et al. 2018) suggest that a single complete defoliation will cause population declines in only a limited number of slow-growing forest and field infestations of pale and black swallow-wort. This contrasts with the released agent *H. opulenta* that will likely have two generations per summer (Weed and Casagranda 2010); it is projected to control more populations of swallow-wort due to the greater amount of damage it can potentially inflict. Agents that have multiple generations, and therefore can provide season-long damage of targeted weeds, are preferred because they limit the ability of plants to recover. This is particularly true for perennial plants (Syrett 1983, Hosking et al. 1994, Winston et al. 2014). As also noted by Milbrath et al. (2019), direct competition between *A. asclepiadis* and *H. opulenta* is likely and should be avoided. For these reasons, *A. asclepiadis* should be considered a low priority agent for release.

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**Literature Cited**


