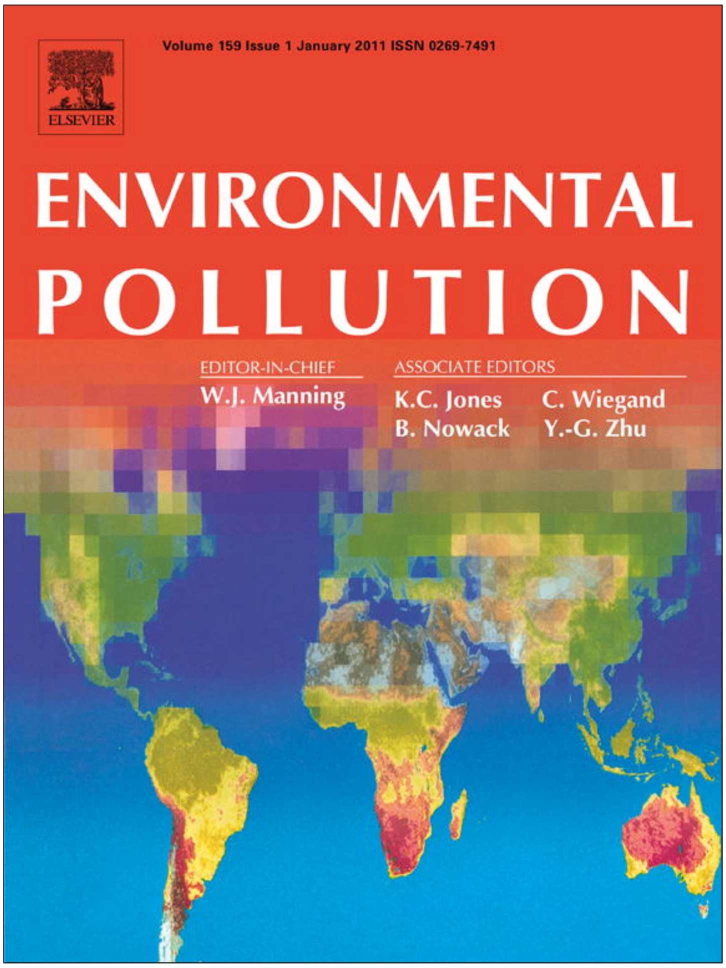
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Effects of herbicides on Behr's metalmark butterfly, a surrogate species for the endangered butterfly, Lange's metalmark

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ABSTRACT

Lange's metalmark butterfly, *Apodemia mormo langei* Comstock, is in danger of extinction due to loss of habitat caused by invasive exotic plants which are eliminating its food, naked stem buckwheat. Herbicides are being used to remove invasive weeds from the dunes; however, little is known about the potential effects of herbicides on butterflies. To address this concern we evaluated potential toxic effects of three herbicides on Behr's metalmark, a close relative of Lange's metalmark. First instars were exposed to recommended field rates of triclopyr, sethoxydim, and imazapyr. Life history parameters were recorded after exposure. These herbicides reduced the number of adults that emerged from pupation (24–36%). Each herbicide has a different mode of action. Therefore, we speculate that effects are due to inert ingredients or indirect effects on food plant quality. If these herbicides act the same in *A. mormo langei*, they may contribute to the decline of this species.

1. Introduction

The Lange's metalmark butterfly, *Apodemia mormo langei* Comstock, is found exclusively in the Antioch Dunes National Wildlife Refuge (ADNWR) in the San Francisco Bay-Delta area, on the southern shore of the San Joaquin River. This refuge, which was opened in 1980, was the first national wildlife refuge in the country that was established to protect endangered plants and insects. Lange's metalmark is one of 15 subspecies of *Apodemia mormo* in the State of California (Opler and Powell, 1962; Powell, 1975; Emmel, 1998). It was listed as an endangered species on June 1, 1976 (Federal Register, 1976) and is in imminent danger of extinction due to the historic loss of its dune habitat by sand mining and by the spread of invasive exotic grasses and forbs which are eliminating naked stem buckwheat (*Eriogonum nudum* var. *auriculatum*), the food plant of the Lange's metalmark butterfly (U.S. Fish and Wildlife Service, 2006).

The population size of the Lange's metalmark at the Antioch Dunes National Wildlife Refuge between 50 and 100 years ago is estimated to have been approximately 25,000 individuals per year. Since that time the degradation and depletion of the unique dunes habitat has resulted in a severe population decline. By 1972, approximately 5000 individuals were present in the dunes (Arnold and Powell, 1983). Since 1997, the population has declined dramatically reaching a low peak count of 45 adults in 2006 (Fig. 1). The host plant for Lange's metalmark butterfly *Apodemia mormo langei* is the perennial, naked stem buckwheat, which grows best in dry, open areas with good drainage. Lange's metalmark uses the buckwheat as a sole food source when in the larval stage and uses the nectar of the flower in the adult stage (U.S. Fish and Wildlife Service, 1984). Arnold and Powell (1983) found a direct positive correlation between the number of mature buckwheat plants at the Refuge and population size of the species.

Invasive plants significantly impact the few remaining acres of habitat at the ADNWR. The naked stem buckwheat plant occupies a limited area within the ADNWR and is threatened with extirpation from this location due to the prolific overgrowth of invasive non-native plants, particularly rip-gut brom (*Bromus diandrus*), vetch (*Vicia villosa*), and yellow starthistle (*Centaurea solstitialis*).

Habitat restoration and the removal of invasive plant species are critical to the recovery of at-risk butterfly populations (New et al., 1995). Several options for removal of invasive weeds exist. These options include hand removal, use of prescribed burns 1997–2001, the application of herbicides, and cattle grazing. The ADNWR has used hand removal to control vetch. Hand removal presents a particular problem because vetch sends out tendrils that wrap around naked stem buckwheat and when removed by hand

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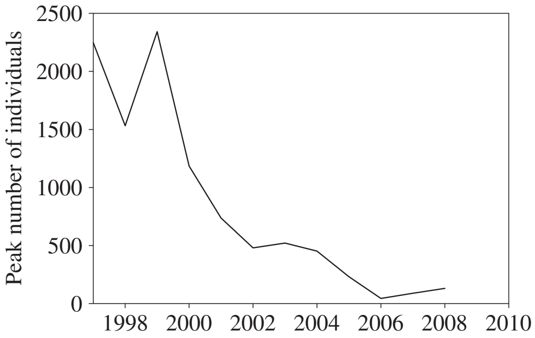

Fig. 1. Population dynamics of Lange's metalmark butterfly at the Antioch Dunes National Wildlife Refuge. Data obtained from USFWS Lange's metalmark surveys.

Table 1
Effects of triclopyr (Garlon 4 Ultra) on Behr's metalmark development and fecundity.

Treatment	$X \pm SE$			
	# pupae produced	# adults produced	Adult longevity (d)	# eggs/female
Control	9.50 ± 0.19*	9.50 ± 0.19*	22.83 ± 2.06	29.92 ± 5.81
Triclopyr	7.25 ± 0.63	7.25 ± 0.63	21.25 ± 2.16	26.11 ± 8.13

*/significantly different based on a *t*-test.

buckwheat plants are often pulled out of the ground as well. In addition, the hand removal of the vetch entrails can scrape eggs and larvae off the stems. Herbicides are a common option for vegetation management and several herbicides are being used in the ADNWR to control invasive weeds. However, there is uncertainty about the ecological risks of herbicides to non-target insects (Freemark and Boutin, 1995; Pratt et al., 1997; Colborn and Short, 1999).

Three of the commonly used herbicides in the ADNWR are triclopyr, sethoxydim, and imazapyr. Triclopyr is a pyridine-based, selective herbicide used to control woody and broad-leaf plants. Triclopyr acts as a plant hormone mimic. Sethoxydim is a selective post-emergence herbicide used to control annual and perennial grass weeds. Sethoxydim is a member of the cyclohexanedione or cyclohexenon family of herbicides. It acts on plants by inhibiting lipid biosynthesis through effects on acetyl-CoA carboxylase. Imazapyr, an imidazolinone herbicide, is a non-selective broad-spectrum, systemic herbicide that works by disrupting protein synthesis.

Although herbicides are designed to kill plants, several studies indicate that some herbicides can adversely affect animal life. For example, studies by Hayes et al. (2003, 2006, 2010) show that atrazine has a negative effect on frogs acting as an endocrine disruptor. Human cell lines have also been shown to be damaged by exposure to atrazine (Fan et al., 2007a,b).

Several insect species have been found to be negatively affected by herbicides (Borkovec et al., 1967; Chio and Sanborn, 1977; Agnello et al., 1986; Eijsackers, 1987; Briust, 1990; Eliyahu et al., 2003; Al-Assiuty and Khalil, 1996). However, the mechanism of how herbicides affect insects is uncertain. Herbicides can affect animals by transforming their food and making it less nutritious and/or by acting directly on physiological systems (Eliyahu et al., 2003; Hayes et al., 2003).

Few studies have been published on the effects of herbicides on butterflies (Russell and Schultz, 2009) and to our knowledge no studies on the effects of herbicides on Lange's metalmark or other metalmark species have been published. Therefore, the objective of this study was to determine whether the three herbicides being used to control weeds in the ADNWR have a negative impact on metalmark butterflies. We used a common related species, Behr's metalmark butterfly *Apodemia virgulti* Behr, for our investigations into the potential toxicity of herbicides to metalmark butterflies.

2. Materials and methods

2.1. Study species

We obtained *A. virgulti* from two sources: Gordon Pratt, University of California, Riverside, CA and Jana Johnson, Moorpark College, Moorpark, CA. The first batch of *A. virgulti* were used to develop methodology used in subsequent studies. The experiments outlined below were all conducted with *A. virgulti* obtained from Jana Johnson, Moorpark College, Moorpark, CA.

2.2. Host plant

Seedlings of Siskiyou Buckwheat, *Eriogonum siskiyouense*, were obtained from Forest Farm Nursery, Williams, Oregon and raised in a greenhouse at Washington State University (WSU) Puyallup. These plants were used to provide food for larvae and oviposition sites for adults.

2.3. Herbicides evaluated

Triclopyr (Garlon 4 Ultra) (Dow AgroSciences LLC, Indianapolis, IN, USA) was obtained from Wilbur Ellis, Auburn, WA. The active ingredient of Garlon 4 Ultra is triclopyr-2-butoxyethyl ester (61.6% active ingredient). Triclopyr acts as an auxin growth regulator. The field rate of Garlon 4 Ultra used in the ADNWR is 18.71 L product/ha.

Sethoxydim (Poast) (BASF Corporation Agricultural Products, Research Triangle Park, NC) was obtained from Wilbur Ellis, Auburn, WA. The active ingredient of Poast is Sethoxydim: 2-[1-(ethoxyimino) butyl]-5-[(2-ethylthio) propyl]-3-hydroxy-2-cyclohexen-1-one (18.0% active ingredient). Sethoxydim acts as a lipid biosynthesis inhibitor. The field rate of Poast used in the ADNWR is 1.75 L product/ha.

Imazapyr (Stalker) (BASF Corporation Agricultural Products, Research Triangle Park, NC) was obtained from Wilbur Ellis, Auburn, WA. The active ingredient is isopropylamine salt of imazapyr (2-[4.5-dihydro-4-methyl-4-(1-methythyl)-5-oxo-1 *H*-imidazol-2-yl]-3-pyridinecarboxylic acid) (27.6% active ingredient). Imazapyr acts as an amino acid inhibitor. The field rate of Stalker applied in the ADNWR is 7.0 L product/ha.

2.4. Herbicide exposure

Three separate experiments were conducted on different dates, one for each herbicide evaluated. Experiment 1, started on February 25, 2008 consisted of exposing 1st instar *A. virgulti* to direct applications of Garlon 4 Ultra (Triclopyr) at the labeled field rate (see above). Experiment 2, started on June 10, 2008 consisted of applying Poast (Sethoxydim) at its labeled field rate. Experiment 3, started on October 28, 2008 consisted of applying Stalker (imazypyr) at its labeled field rate (see above).

Each product was applied directly to batches of 10 1st instar *A. virgulti* and 0.22 ± 0.003 g of their food, *E. siskiyouense* contained in 5 cm petri plates. Each experiment was replicated four times. We were only interested in the effects of field rate of each product on butterfly health. Therefore, applications of recommended field rates were made with a Potter Tower (Burkard Scientific, Uxbridge, UK) set at

Table 2
Effects of triclopyr (Garlon 4 Ultra) on morphology of Behr's metalmark butterfly.

Treatment	$X \pm SE$						
	Pupal length (mm)	Pupal weight (mg)	Adult body weight (mg)	Adult abdomen width (mm)	Adult abdomen length (mm)	Adult body length (mm)	Diagonal wing length (mg)
Control	10.50 ± 0.18	0.087 ± 0.004*	0.05 ± 0.006	2.55 ± 0.09	5.57 ± 0.13*	10.43 ± 0.20	9.11 ± 0.21
Triclopyr	11.36 ± 0.15	0.100 ± 0.005	0.05 ± 0.005	2.57 ± 0.15	6.05 ± 0.20	11.09 ± 0.29	9.56 ± 0.23

*/significantly different based on a *t*-test.

Table 3
Effects of sethoxydim (Poast) on Behr's metalmark development and fecundity.

Treatment	X ± SE			
	# pupae produced	# adults produced	Adult longevity (d)	# eggs/female
Control	8.87 ± 1.13*	8.87 ± 1.13*	28.63 ± 3.20	25.36 ± 3.44
Sethoxydim	6.50 ± 1.29	6.50 ± 1.29	24.95 ± 4.16	25.91 ± 2.70

*/significantly different based on a *t*-test.

Table 5
Effects of imazapyr (Stalker) on Behr's metalmark development and fecundity.

Treatment	X ± SE			
	# pupae produced	# adults produced	Adult longevity (d)	# eggs/female
Control	8.25 ± 0.25*	8.25 ± 0.25*	23.93 ± 2.20	44.00 ± 6.13*
Imazapyr	5.25 ± 0.63	5.25 ± 0.63	24.12 ± 3.48	20.50 ± 6.87

*/significantly different based on a *t*-test.

68.95 kPa. The Potter Tower was calibrated to apply each herbicide at its respective field rate in 187 L water–herbicide solution/ha. Controls were exposed to water only applied in the same way as described for the herbicides. After application of herbicides, the larvae and host plants were allowed to dry for 5 min. A glass cover was then placed over the petri plates and they were transferred to a freestanding environmental chamber set at 25 ± 0.1 °C, 50% relative humidity, and a 16:8 dark–light regimen. Shortly after exposing larvae and buckwheat to the herbicides, we sprayed a batch of 10 buckwheat plants once with the same rate of herbicide or water (controls) listed above with a Solo backpack sprayer set at 207 kPa. These plants were kept in a greenhouse under natural light and temperature and were fed to larvae after the initial herbicide-treated buckwheat was consumed. New food plants were introduced into the petri dishes every other day until the larvae had pupated.

The survival of larvae was recorded daily. When pupation occurred, pupae were weighed and moved to small plastic cups (3 cm diameter, 3.5 cm depth). Emerged adults were sexed and one female and one male were transferred to oviposition chambers consisting of plastic cups (6.5 cm diameter, 12.5 cm depth). Buckwheat plants (ca 15 cm) were placed through a hole in the bottom of the cups and these cups were inserted into second smaller cups (5 cm diameter, 10 cm depth) containing water such that the buckwheat plant root system had a continuous supply of water. The chamber was covered with a fine mesh screen to contain the butterflies. A cotton ball soaked in a water–honey solution was also placed in the oviposition chambers as a food source for the adults. The number of eggs laid over a female's lifespan was recorded.

The following morphological measurements were also recorded: pupal length (mm), pupal weight (mg), adult body length (mm), adult abdomen width (mm), diagonal hind wing length (mm), and adult body weight (mg).

2.5. Statistical analysis

All data were analyzed with a *t*-test (SAS Institute, 2002–2003). A Mann–Whitney Rank Sum Test (nonparametric *t*-test) for pupal weight and adult abdomen length was used in the triclopyr experiment because the assumption of normality was not met.

3. Results

3.1. Triclopyr

Exposure of *A. virgulti* to triclopyr resulted in significant reductions in the number of pupae produced ($t = 4.47$, $df = 10$, $p = 0.001$) and thus the number of adults that emerged from pupation ($t = 4.47$, $p = 0.001$) compared to the control (Table 1). Adult emergence was 24% lower in the treatment compared to the control. Furthermore, the weight of pupae was significantly higher in individuals exposed to triclopyr ($T = 1139.5$, $p = 0.05$) (Table 2). Adult abdomen length was significantly longer ($T = 680.5$, $p = 0.048$) in individuals exposed to triclopyr compared to the control ($T = 1139.5$, $p = 0.05$). All other parameters measured in this study were unaffected by exposure to triclopyr.

3.2. Sethoxydim

Exposure of *A. virgulti* to sethoxydim resulted in significant reductions in the number of pupae produced ($t = 3.29$, $df = 10$, $p = 0.008$) and therefore the number of adults that emerged from pupation ($t = 3.29$, $p = 0.008$) compared to the control (Table 3). Adult emergence was 27% lower in the treatment compared to the control. Sethoxydim had no significant affect on the other parameters measured in this study (Table 4).

3.3. Imazapyr

Exposure of *A. virgulti* to imazapyr resulted in a significant reduction in the number of pupae produced ($t = 4.43$, $df = 10$, $p = 0.004$) and therefore the number of adults produced ($t = 4.43$, $df = 10$, $p = 0.004$) compared to the control (Table 5). Adult emergence was 36% lower in the treatment compared to the control. The other parameters measured in this study were not significantly affected by exposure to imazapyr (Table 6).

4. Discussion

Results of the present study show that all three of the herbicides evaluated significantly reduced adult emergence of *A. virgulti* (24–36%) after exposure to field rates. Therefore these herbicides may have a negative effect on butterflies in the field. It is important to note that if the butterflies made it to the pupal stage, they would emerge to the adult stage. In other words, herbicide exposure reduced the number of individuals reaching the pupal stage. Adult emergence was actually 100% from all pupae produced. However, the end result is a reduction in the number of adults and these adults contribute to the next generation. Thus, a reduction in the number of adults will have a negative impact on populations of this species.

Russell and Schultz (2009) evaluated the effects of two herbicides, fluazifop-p-butyl and sethoxydim and a surfactant on two butterfly species, *Icaricia icarioides blackmorei* and *Pieris rapae*. They found that survivorship of *P. rapae* was reduced by 32% after exposure to sethoxydim and 21% after exposure to fluazifop-p-butyl. However, neither herbicide affected survivorship of *I. i. blackmorei*. Wing size and pupal weights of male and female *P. rapae* were reduced after exposure to both herbicides. Furthermore, an average 21% reduction in development time was manifested in *I. i. blackmorei* by both herbicides. The reductions in

Table 4
Effects of sethoxydim (Poast) on morphology of Behr's metalmark butterfly.

Treatment	X ± SE						
	Pupal length (mm)	Pupal weight (mg)	Adult body weight (mg)	Adult abdomen width (mm)	Adult abdomen length (mm)	Adult body length (mm)	Diagonal wing length (mg)
Control	10.91 ± 0.10	0.084 ± 0.012	0.05 ± 0.003	2.53 ± 0.11	5.63 ± 0.11	10.69 ± 0.13	10.11 ± 0.13
Sethoxydim	10.59 ± 0.12	0.097 ± 0.016	0.05 ± 0.003	2.62 ± 0.15	5.68 ± 0.17	10.70 ± 0.17	9.84 ± 0.14

*/significantly different based on a *t*-test.

Table 6
Effects of imazapyr (Stalker) on morphology of Behr's metalmark butterfly.

Treatment	$X \pm SE$						
	Pupal length (mm)	Pupal weight (mg)	Adult body weight (mg)	Adult abdomen width (mm)	Adult abdomen length (mm)	Adult body length (mm)	Diagonal wing length (mg)
Control	11.07 ± 0.17	0.091 ± 0.004	0.05 ± 0.003	2.64 ± 0.15	5.70 ± 0.14	10.40 ± 0.18	10.36 ± 0.19
Imazapyr	10.91 ± 0.21	0.088 ± 0.005	0.05 ± 0.005	2.79 ± 0.21	5.82 ± 0.23	10.29 ± 0.28	10.39 ± 0.27

*/significantly different based on a *t*-test.

survivorship seen by Russell and Schultz (2009) are similar to those found in our study. We found that *A. virgulti* survival to the adult stage was reduced 27% after exposure to sethoxydim.

Although it has been shown that certain herbicides can have negative effects on animal life, the three herbicides we evaluated have completely different modes of action. Therefore we speculate that the effects exhibited on pupal development are not due to the active ingredients, but rather to 1) an inert ingredient or combination of inert ingredients contained within the formulations of these products and/or 2) indirect effects on the food plant quality.

Herbicides are used in public and private lands to reduce and/or eradicate invasive plant species and thus enhance habitat for at-risk butterflies. As such, herbicides are important tools for habitat management. However, the loss of even a few individuals can decrease the overall breeding population (New, 1991). Therefore herbicide use in endangered butterfly habitat should be carefully considered by weighing all risks and benefits. As herbicide use increases in natural areas, managers need to address the potential likelihood that non-target organisms will be exposed and perhaps negatively affected. Designing conservation strategies for sites that harbor rare butterflies is challenging because management actions to control invasive species must maximize their impact on problematic species while limiting their impact on native species, especially threatened and endangered species.

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