

Seasonal Adaptations to Day Length in Ecotypes of *Diorhabda* spp. (Coleoptera: Chrysomelidae) Inform Selection of Agents Against Saltcedars (*Tamarix* spp.)

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ABSTRACT Seasonal adaptations to daylength often limit the effective range of insects used in biological control of weeds. The leaf beetle *Diorhabda carinulata* (Desbrochers) was introduced into North America from Fukang, China (latitude 44° N) to control saltcedars (*Tamarix* spp.), but failed to establish south of 38° N latitude because of a mismatched critical daylength response for diapause induction. The daylength response caused beetles to enter diapause too early in the season to survive the duration of winter at southern latitudes. Using climate chambers, we characterized the critical daylength response for diapause induction (CDL) in three ecotypes of *Diorhabda* beetles originating from 36, 38, and 43° N latitudes in Eurasia. In a field experiment, the timing of reproductive diapause and voltinism were compared among ecotypes by rearing the insects on plants in the field. CDL declined with latitude of origin among *Diorhabda* ecotypes. Moreover, CDL in southern (<39° N latitude) ecotypes was shortened by more than an hour when the insects were reared under a fluctuating 35–15°C thermoperiod than at a constant 25°C. In the northern (>42° N latitude) ecotypes, however, CDL was relatively insensitive to temperature. The southern ecotypes produced up to four generations when reared on plants in the field at sites south of 38° N, whereas northern ecotypes produced only one or two generations. The study reveals latitudinal variation in how *Diorhabda* ecotypes respond to daylength for diapause induction and how these responses affect insect voltinism across the introduced range.

KEY WORDS Critical daylength response, voltinism, biological control, biological invasions

Herbivorous insects used for the biological control of weeds often experience novel variation in climate, daylength, and host plant qualities in the introduced range (Drea 1991). Inadequate matching of insect agents and the host environment is often the cause of poor or failed local establishment during biological

control implementation (Crawley 1989, Julien and Griffiths 1999). Such discontinuity may partly explain why only about one third of biological control programs have achieved successful control of invasive weed species (Crawley 1989, Denoth et al. 2002). Close synchrony between the agent and its target host plant is particularly important for managing perennial weeds that require multiple defoliation events (Drea 1991, Center et al. 2000, Tipping et al. 2008). Perennial plants often compensate for herbivore attack by regenerating when the herbivore is absent, such as when an herbivorous insect enters winter diapause before the deciduous host plant is dormant.

Models that match climates between the source region and the target point for introduction have been used to increase the likelihood of successful establishment of biological control agents (Sutherst 2003, Goolsby et al. 2005, Rafter et al. 2008). Climate features, however, only partially describe the physiological constraints of introduced organisms. Seasonal adaptations to daylength may also affect the establishment (Miller et al. 2000, Velarde et al. 2002, Lewis et al. 2003). Photoperiod (daylength) is one of the most important environmental cues used by

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herbivorous insects to synchronize their life cycles with the seasonal climate and host plant conditions (Beck 1980, Tauber et al. 1986, Danks 1987). Because daylength is a function of latitude, there are often latitudinal variations in how insects respond to this environmental factor (Bradshaw 1976, Masaki 1999). Consequently, when insects are introduced into environments with a different daylength regime, the insect life cycle may become less synchronized with climate and host plant season, resulting in poor survival and control efficacy (Lewis et al. 2003). Introductions of biological control insects may thus provide unique opportunities to study how insects are adapted to track seasonal environmental variation and how insect life cycles may change in response to novel conditions.

Saltcedars (*Tamarix* spp.) comprise an invasive group of weeds in North America ranging from northern Mexico to Montana and from Kansas to coastal California (Horton 1977). The plants were originally introduced from Eurasia and several species and hybrids of *Tamarix* are involved in the invasion (Baum 1978, Gaskin and Schaal 2003). Almost one million hectares of land are infested by *Tamarix* spp. across the western U.S., including rangelands, agricultural areas, and riparian zones that provide habitats for endangered species (Shafroth et al. 2005). Many efforts have been undertaken in recent decades to control saltcedars because of its numerous environmental and economic impacts (Barrows 1998, Zavaleta 2000, McDaniel and Taylor 2003).

The leaf beetle *Diorhabda carinulata* (Desbrochers) (Coleoptera: Chrysomelidae), formerly *D. elongata* (Brullé) *sensu lato*, was introduced into North America for the biological control of saltcedars (Lewis et al. 2003, Tracy and Robbins 2009). The beetles were originally collected near the town of Fukang in the Xinjiang Province in China (latitude 44.17° N, longitude 87.98° E, elevation 567m) and the first open-releases took place in 2001. The Fukang beetles successfully established at several sites north of 38° N latitude in North America (e.g., in Nevada, Colorado, and Wyoming), with hundreds to thousands of hectares of saltcedar defoliated within 5 yr at some sites (DeLoach et al. 2004). However, the beetles failed to establish south of 38° N (Lewis et al. 2003). It was shown that the Fukang beetles cease reproduction and enter reproductive diapause when daylength is 14 h and 39 min or less under field conditions (Bean et al. 2007a). This means that at latitudes south of 36° N, where the longest days of spring and summer never exceed 14 h 39 min, the Fukang beetles are constrained to a univoltine life cycle and enter reproductive diapause in the summer when warm temperatures deplete metabolic reserves and inhibit the capability to survive until the following spring (Lewis et al. 2003, Bean et al. 2007a). At latitudes north of 38° N, the critical photoperiod responses of the Fukang beetles allow a second (Lewis et al. 2003, Bean et al. 2007a), or even a third generation (Li et al. 2000). Pushing diapause induction later into the season allows beetles to be multivoltine, feed later in the season

and defoliate *Tamarix* multiple times per year. Several defoliation events may reduce plant performance more than just one event per year (Bacher and Schwab 2000, Wolfe 2002, Newington and Callaway 2006).

To overcome the lack of establishment of Fukang beetles south of 38° N, other ecotypes from the *Tamarix*-feeding species group within the genus *Diorhabda* have been collected from latitudes further south in Eurasia (Tracy and Robbins 2009). The southern ecotypes, named after the original site or region where the beetles were collected, include Turpan (*D. carinulata* from China, Xinjiang Province, latitude 42.86° N, longitude 89.22° E, elevation 70 m above sea level), Karshi (*D. carinata* (Faldermann) from Uzbekistan, latitude 38.86° N, longitude 65.72° E, elevation 350 m) and Crete (*D. elongata* Brullé) from Greece, latitude 35.83° N, longitude 24.60° E, elevation 10 m) (Milbrath et al. 2007, Tracy and Robbins 2009). For the purpose of this paper we will refer to the beetles as different ecotypes of *Diorhabda*. All four ecotypes are specialized to feed on the foliage of *Tamarix* spp. (Milbrath and DeLoach 2006).

The current study investigates the relationship between critical daylength responses for diapause induction and voltinism in four ecotypes of *Diorhabda* beetles (Fukang, Turpan, Karshi, and Crete) that are intended for release in the United States. The critical daylength response for diapause induction (CDL) is a genetically determined characteristic of a population and is defined as the daylength when 50% of the population enters reproductive diapause (Beck 1980, Saunders 2002). In the first experiment, we characterized CDL in the three ecotypes Turpan, Karshi, and Crete in the laboratory. Based on previous studies on the Fukang ecotype, our hypothesis was that these southern ecotypes would have shorter CDL, allowing multivoltine life cycles south of 38° N. Using the defined CDL from the laboratory study, we then predicted the time window when the beetles would be reproductively active based on the daylength regime at a given study site. In a field experiment, we tested these predictions by studying the timing of reproductive diapause and voltinism under seminatural conditions by experimentally rearing the four *Diorhabda* ecotypes on plants inside sleeve-cages.

Materials and Methods

Insect Cultures. The Fukang beetles used in the field experiment came from a field population near Lovelock, NV (latitude: 40.26° N, longitude: 118.44° W, elevation: 1213 m) in the United States. This population had been released in 2001 and showed substantial population expansion in the subsequent years. The stock cultures of the other ecotypes (Turpan, Karshi, and Crete) used in the experiments were maintained as previously described (Bean et al. 2007b) on *T. ramosissima* foliage at the Palisade Insectary, Colorado Department of Agriculture, CO. They were first imported from the foreign collection sites to the

Table 1. Study sites included in field exp

Site	Latitude	Elevation (m)	Climate	<i>T. ramosissima</i> Foliage green
Arkansas River, Pueblo, CO	38.2°N	1,460	Interior, semi-arid	May–Sept.
Kern NWR, Delano, CA	35.5°N	70	Interior/coastal	April–Oct.
Canadian River, Borger, TX	35.4°N	950	Interior, semi-arid	April–Oct.
Mojave River, Barstow, CA	34.9°N	550	Desert, arid	Mar.–Nov.
Santa Clara River, Santa Paula, CA	34.3°N	Sea level	Coastal	Feb.–Dec. ^a

^a Partly evergreen.

USDA-ARS Exotic and Invasive Weeds Quarantine Facility, Albany, CA in 2002.

Diapause Induction: CDL. CDL in the Fukang ecotype has been described previously (Bean et al. 2007a). In this experiment, we characterized CDL in the three ecotypes Turpan, Karshi, and Crete at a constant 25°C temperature in the laboratory. Methods for estimating CDL are described in detail in Bean et al. (2007a) and summarized below. Insects taken from the stock cultures at the Palisade Insectary were reared from eggs or first instar larvae under conditions of controlled temperature and photoperiod in growth chambers (Hotpack, Warminster, PA, or model I30BLL, Percival, Perry, IA). Test photoperiods included 10, 12, 13, 13.5, 14, 14.5, and 15 h of light, with each *Diorhabda* ecotype tested under at least four photoperiod treatments. The sensitive period for diapause induction extends into the adult stage (Bean et al. 2007b). The insects were therefore reared from eggs or first instars until adulthood under test photoperiods. Larvae were mass reared in groups of at least 20, transferred to sand for pupation and newly emerged adults were paired and given fresh foliage. The number of pairs tested at each condition ranged from 25 to 52 with most conditions having at least 30 pairs. Paired adults were kept under experimental conditions for 15–20 d and if no oviposition occurred the insects were scored as in diapause. Under conditions that promote continuous development, 100% of females oviposit within the first 8 d after adult emergence (Bean et al. 2007b). Females that had not oviposited were dissected to confirm that they were in diapause (Bean et al. 2007b).

In a second experiment, we used a thermoperiod that had an average temperature of 25°C and fluctuated between 9 h at 35°C and 9 h at 15°C. The purpose was to investigate if fluctuating temperature, as would occur under field condition, can modify photoperiodic responses. We used photoperiod treatments that equaled or were shorter than the CDL at a constant 25°C. The Turpan ecotype was tested under 13.5 and 14 h of light, Karshi under 12, 13, and 14 h of light, whereas the Crete ecotype could only be tested under one photoperiod treatment (13 h of light) because of shortage of beetles. Chambers were programmed to make a gradual transition between the high and low temperatures over a 3 h period. The warming phase of the thermoperiod began at lights on and the cooling phase started 12 h later with the temperature dropping to 15°C after 3 h. Under most photoperiods, the lights

went out during the cooling phase. This pattern was selected to keep thermoperiods constant, to keep average temperature at 25°C, and to keep the relationship between temperature and light cycle matched with naturally occurring temperature patterns in which temperatures rise after sunrise and begin to fall before sunset. The temperature range in the thermoperiod experiment was chosen based on a previous study in which the same 15–35°C treatment was used for the Fukang population, but also to simulate the average high and low July temperatures in parts of the interior western U.S. where *Tamarix* spp. are found (Bean et al. 2007a).

Critical daylength was estimated and compared among *Diorhabda* ecotypes using logistic regression (PROC GENMOD, binomial, logit; SAS Institute 2008). Reproductive status was the binary response (1 for diapause, 0 for reproductive) and hours of light the independent variable. Inverse prediction was used to calculate the daylength at which there is a 0.50 probability of diapause (critical daylength) plus 95% CL.

Volinism and Timing of Diapause in the Field. In the field experiment, the 4 ecotypes (Fukang, Turpan, Karshi, and Crete) were reared separately in nylon mesh sleeves on the same caged plants, from spring until all ecotypes stopped reproducing. The number of generations was measured and the initiation of diapause was estimated. The experiment was completed at 5 study sites (Table 1). All sites were heavily infested by *Tamarix ramosissima* or its hybrid forms and are intended release sites of *Diorhabda* beetles.

All 4 ecotypes were exposed to the same cold temperatures and short daylength conditions in the laboratory during the winter 2006/2007 to ensure that they would have experienced and terminated reproductive diapause before the start of the experiment (Bean et al. 2007b). In spring 2007, before *Tamarix* budburst, 3 experimental trees at each study site were selected and pruned to fit within 2 × 2 × 2 meter nylon mesh cages. To produce first instar larvae for introducing in experimental cages, adult beetles (12 specimens per ecotype), were placed into 100 × 75 cm mesh sleeves on 2 of the experimental trees (6 beetles per sleeve) at each site in the early spring, when the trees had started to produce foliage. Because the timing of *Tamarix* bud burst varied among study sites, the starting dates varied although all ecotypes were started at the same date at a given site. We assumed that timing of adult emergence in the spring would be similar among ecotypes, around or just after the timing

Table 2. Results of logistic regression (binomial, logit) investigating the effects of day length and ecotype on diapause incidence in *Diorhabda* leaf beetles

Effect	χ^2	df	P
Ecotype (E)	40.59	2	<00.001
Day-length (DL)	287.63	1	<00.001
E × DL	37.59	2	<00.001

of *Tamarix* bud burst in the spring, which is commonly observed in Chrysomelidae (Fernandez and Hilker 2007).

Experimental branches were examined to remove potential predators and other herbivores before the sleeve including adult beetles was attached to the plants. The adults were left to feed and oviposit inside the cages for 3 wk, or until enough larvae had been produced to initiate three larval sleeves with ≈75–100 larvae per sleeve and ecotype. Each ecotype was replicated 3 times at each study site, on 3 individual *Tamarix* trees. When enough larvae had been produced, the adult beetles were collected to determine reproductive condition (reproductively active or diapause).

Each study site was visited at least every other week until all ecotypes had stopped reproducing. The objective was to ensure that enough larvae (≈40 individuals) for each ecotype and generation would develop until adulthood. Twenty newly emerged adult beetles were left to reproduce. Excess adults were collected for dissections to confirm reproductive status. Pupae and larvae in the sleeves were counted at each site visit. If the experimental branch was wilting, or if there seemed to be a risk that larvae would consume all foliage, the sleeve including larvae was moved to another branch. To move sleeves, the side shoots of the old branch containing foliage and larvae were broken off and carried inside the sleeve to a new branch on the same tree. The sleeve including larvae was then sealed onto the new branch. Larvae were observed to move onto the new shoot with fresh foliage. If there was an excess of larvae (>150–200 individuals), some were removed to avoid over-consumption of foliage. We also continuously removed predators and other herbivores. Perlite was added to each sleeve to be used as a pupation substrate.

This protocol was repeated until no eggs and larvae had been produced by the adult generation within 4 weeks, the assumption being that the adults were in diapause. These adults were also collected to confirm their reproductive status.

Results

Diapause Induction: CDL. Diapause incidence in *Diorhabda* was affected by daylength and varied among ecotypes (Table 2). Regression analyses revealed that the Turpan ecotype had the longest CDL; the Karshi ecotype had an intermediate CDL, whereas the Crete ecotype had the shortest CDL (Table 3).

In the thermoperiod experiment, the percentage of females in diapause for the Turpan ecotype was 97%

Table 3. Critical day lengths for diapause induction in three *Diorhabda* ecotypes

Ecotype	Critical day length ^a	95% confidence interval ^b
Turpan	14 h 07 min	14 h 02 min–14 h 13 min
Karshi	13 h 46 min	13 h 36 min–14 h 02 min
Crete	13 h 16 min	12 h 48 min–13 h 38 min

^a Critical day length calculated using logistic regression.

^b The 95% confidence intervals were calculated for the 50% diapause point.

(*n* = 36) under 13 h 30 min hours of light, and 51% (*n* = 37) under 14 h of light. The results for the Karshi ecotype, including a comparison with results from the constant temperature treatment described above, are presented in Fig. 1. The critical daylength response for diapause induction under the thermoperiod treatment for the Karshi ecotype was estimated to be 12 h and 19 min. The Crete ecotype was only tested under one daylength treatment in the thermoperiod experiment; 13 h (0% in diapause, *n* = 39 pairs).

Voltinism and Timing of Diapause in the Field. Based on the CDL characterized at constant 25°C in the laboratory for the Fukang ecotype in Bean et al. (2007a), and for Turpan, Karshi, and Crete in the current study, we used the daylength at each field site to predict the dates when the different *Diorhabda* ecotypes would be reproductively active. The intervals during which day lengths are longer than the CDL are highlighted in gray for each ecotype at each study site (Fig. 2a–d). The result is a predicted time-window during which >50% of beetles are expected to be reproductive. Reproductive diapause in *Diorhabda* is terminated during the winter and the life cycle is therefore at least univoltine (Bean et al. 2007b). The predicted time-windows shown in Fig. 2 therefore indicate the possibility of multivoltine life cycles.

The Fukang ecotype had a univoltine life cycle at the southernmost study sites, Santa Clara River (34.3° N) and Mojave River (34.9° N) (Fig. 2a). Adult F1 beetles (first generation adults) emerging by mid-June at Mojave River, and in the beginning of August at Santa Clara River, were in reproductive diapause, which was confirmed by dissections (100% in diapause; *n* = 38 specimens). The Fukang beetles had a bivoltine life cycle at the study sites further north at Canadian River (35.4° N), Kern National Wildlife Refuge (Kern NWR; 35.5° N), and Arkansas River (38.2° N) (Fig. 2a). At Kern NWR, 83% of F1 adults were in reproductive diapause when collected 13 June (*n* = 14), and 100% of F2 adults collected in September were in diapause (*n* = 40). At Arkansas River, the first generation adults (F1) collected in late July were all reproductively active (0% in diapause, *n* = 20), whereas in late August, 25% of F2 adults were in diapause and the remaining beetles were in a reproductive stage that could not be categorized as either diapause or reproductive (Stage 1–3 described in Bean et al., 2007b; *n* = 20 specimens in total). It is reasonable to assume that they were in diapause because the beetles did not oviposit in the field.

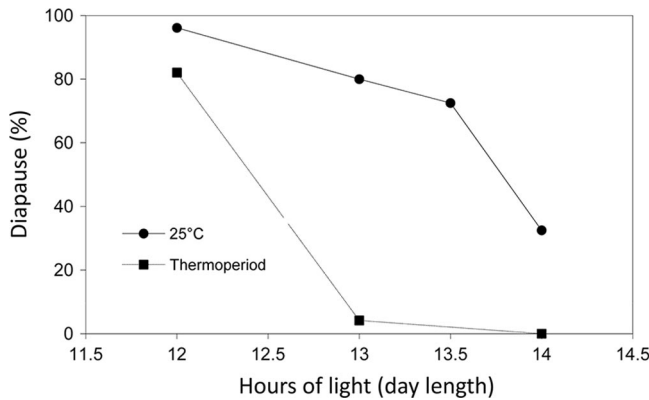


Fig. 1. Diapause incidence in the Karshi ecotype of *Diorhabda* beetles under different day lengths (hours of light). Temperatures for the thermoperiod experiment fluctuated between 35°C during the photophase and 15°C during the scotophase with an average of 25°C. The constant temperature experiment was run at 25°C. The number of females analyzed varied between 29 and 40 per treatment.

The Turpan ecotype had a bivoltine life cycle at all study sites (Fig. 2b). However, we were not able to test the Turpan beetles at the northernmost site (Arkansas River) because of a shortage of beetles. Furthermore, because few adult F1 beetles emerged in the field, there were insufficient numbers of adult beetles to estimate the proportion of beetles in diapause. Instead, we used all F1 adults for testing in the field and to initiate another generation. For the second generation (F2), we received enough adults to be able to estimate proportion in diapause. For those F2 adults collected 4–24 September at Santa Clara River ($n = 34$), Kern NWR ($n = 19$), and Canadian River ($n = 28$), all (100%) were in reproductive diapause.

The Karshi ecotype produced 4 generations at Mojave River, three generations at Santa Clara River, Canadian River and Kern NWR, and 2 generations at the northernmost site, Arkansas River (Fig. 2c). Of the Karshi beetles emerging in August at sites south of 37° N latitude, 0% were in diapause ($n = 23$ collected 23 August–28 at Canadian River), whereas at the northernmost site, Arkansas River, 5% were in diapause by mid-August (F1 adults; $n = 19$ collected 10 August). In late September and beginning of October, 30–45% were in diapause at Mojave River (F3 adults; $n = 20$ collected 17 September, $n = 20$ collected 2 October), 45% in diapause at Santa Clara River (F2 adults; $n = 20$ collected 28 September, $n = 20$ collected 15 October). Further north, 100% of the Karshi beetles were in diapause by early October (F3 adults; $n = 17$ collected 8 October at Canadian, and F2 adults; $n = 20$ collected 10 October at Arkansas River). In November, when the last generation of adult beetles was emerging at Mojave (F4 adults) and Santa Clara (F3 adults), 100% were in diapause ($n = 25$). Most experimental plants still had green foliage present at this time.

The Crete ecotype had a trivoltine (Mojave and Canadian Rivers) or bivoltine (Santa Clara River and Kern NWR) life cycle at our field sites south of 37° N latitude (Fig. 2d). At the northernmost site (Arkansas

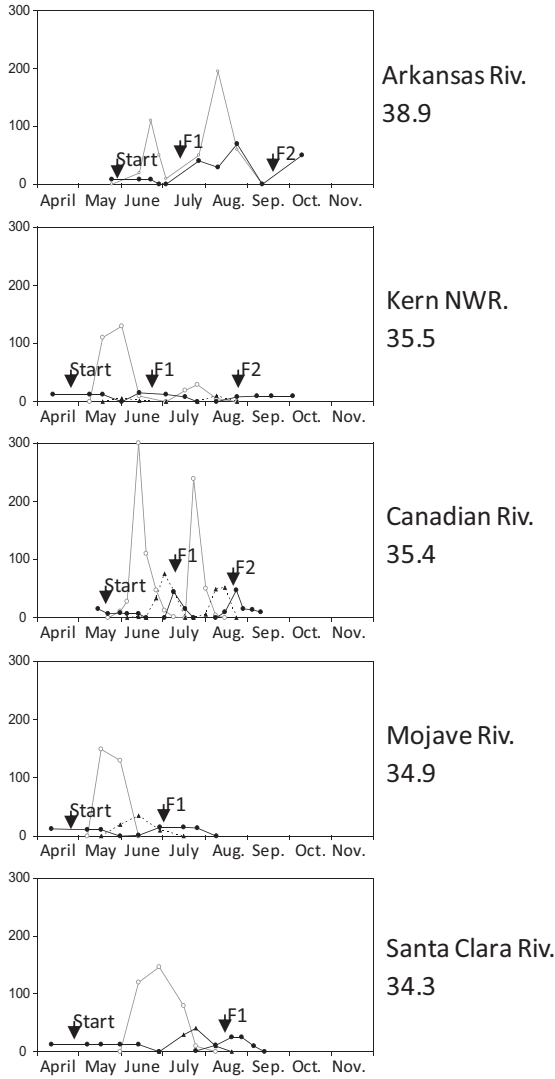
River), the Crete ecotype was bivoltine (Fig. 2d). The Crete beetles entered reproductive diapause by late September or beginning of October at the southernmost sites (Santa Clara and Mojave Rivers: 0% in diapause by early September [F1 and F2 adults; $n = 25$ collected 6 September–13], and 22% in diapause at Mojave by early October [F2 adults; $n = 6$ collected 2 October]). At Canadian River, 8% of the Crete beetles were in diapause by late August (F1 adults; $n = 47$ collected 23 August–28), and 63% in diapause by mid-October (F2 adults; $n = 16$ collected 16 October). At Arkansas River, 25% of the Crete beetles were in diapause by late August (F1 adults; $n = 20$ collected 24 August), and 100% in diapause by October (F2 adults; $n = 20$ collected 10 October).

Discussion

The laboratory-based experiments revealed variation in CDL among three ecotypes of *Diorhabda* beetles that reflected their latitude of origin. The northernmost ecotype from Turpan, China (42.9° N latitude) had the longest CDL, the mid-latitude ecotype from Karshi, Uzbekistan (38.9° N latitude) had an intermediate CDL, and the southernmost ecotype from Crete, Greece (35.8° N latitude) had the shortest CDL (Table 3). These are all shorter than the CDL of the Fukang ecotype (44.1° N latitude), which was 15 h 8 min under constant 25°C in the laboratory (Bean et al. 2007a).

When the different ecotypes were tested under field conditions along a latitudinal gradient, the timing of reproductive diapause and voltinism corroborated the laboratory results. At our five study sites ranging from 34.3° N to 38.2° N latitude, we found that the two southern ecotypes, Karshi and Crete, always produced more (3 to 4), or the same (2), number of generations compared with the 2 northern Chinese ecotypes, Turpan (2 generations) and Fukang (1 or 2 generations). At our northernmost site, Arkansas River (38.2° N latitude) where a Fukang field population usually pro-

a Fukang (*D. carinulata*)



b Turpan (*D. carinulata*)

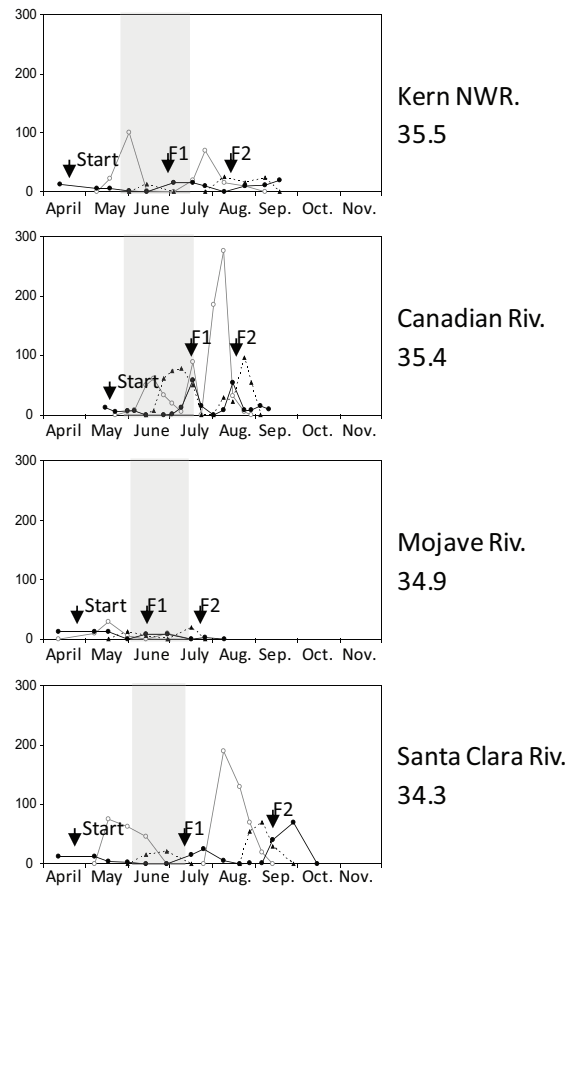


Fig. 2. (a–d) Results from field experiments where the timing of reproductive diapause and voltinism (number of generations) of four ecotypes of *Diorhabda* beetles were studied at five sites in western United States. (a) Fukang, *D. Carinulata*; (b) Turpan, *D. carinulata*; (c) Karshi, *D. Carinata*; and (d) Crete, *D. elongata*, ecotypes. Solid black circles with solid lines represent adult beetles (Start and F1–F4 generations), open gray circles with gray lines represent larvae, and solid black triangles with dotted lines represent pupae. Gray areas inside the graphs highlights the time period when daylength is longer than the critical daylength for diapause induction (CDL) of a given ecotype at a given site.

duced 2 generations per year (D. W. Bean and D. Eberts, unpublished data), we found that all 4 ecotypes completed two generations before diapause. Although the timing of reproductive diapause varied among study sites and ecotypes, all ecotypes were able to complete development before the plants started to senesce. It is therefore reasonable to assume that those last-generation adults would have become the overwintering generation under normal field conditions.

The variation in critical daylength responses detected among *Diorhabda* ecotypes is consistent with previous studies showing similar latitudinal clines in

photoperiodic responses for diapause induction in insects (Riedl and Croft 1978, Solbreck and Sillén-Tullberg 1981, Masaki 1999, Demont and Blanckenhorn 2008). The field experiments measuring the timing of diapause and voltinism produced, however, some unexpected results. For example, predictions based upon the CDL described in Bean et al. (2007a) suggested that the Fukang beetles should have a univoltine life cycle at our study sites south of 38° N, and that bivoltinism should be expected mainly at our northernmost site on the Arkansas River in Colorado (Lewis et al. 2003). In our study, the Fukang beetles had a bivoltine

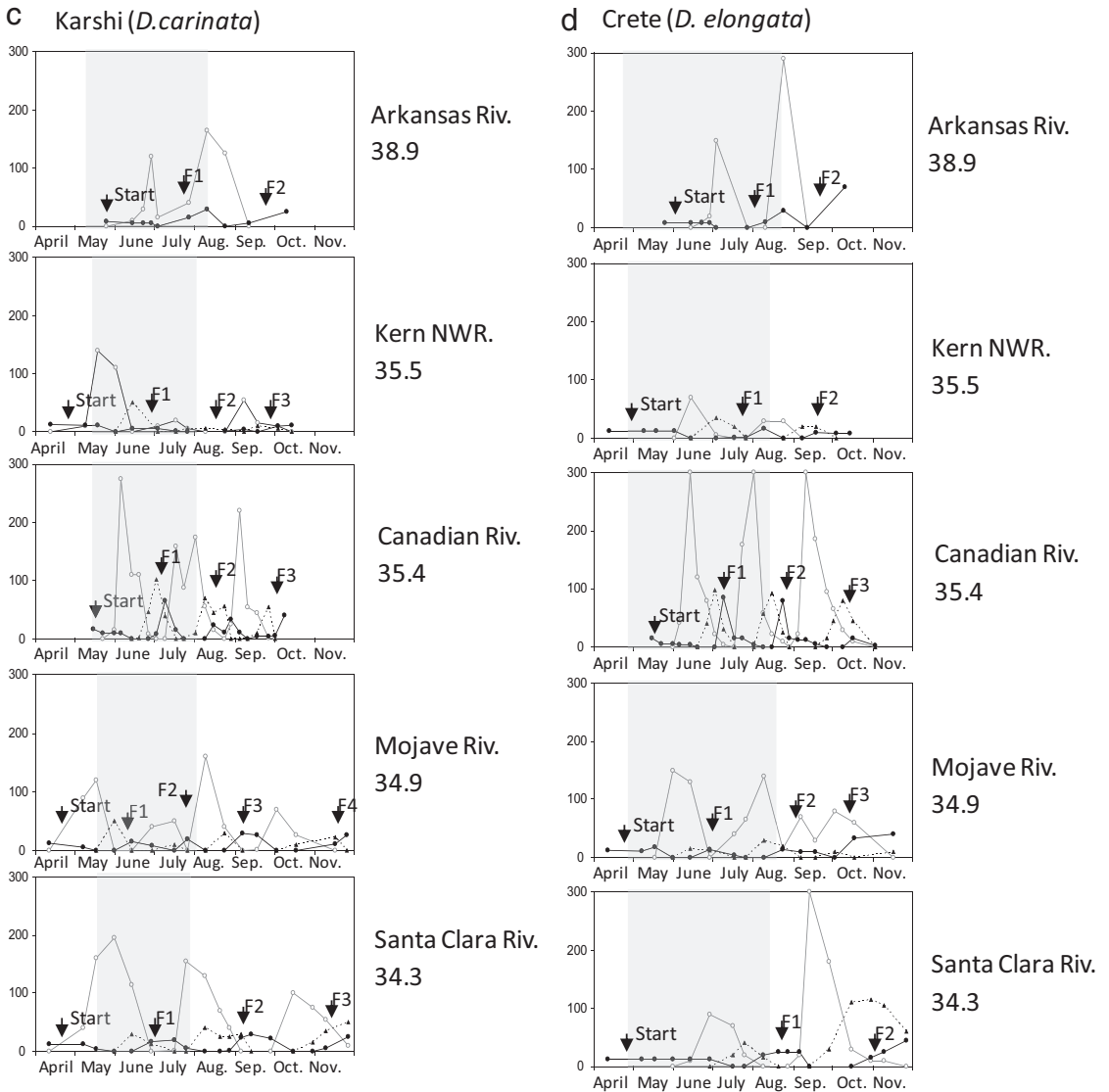


Fig. 2. Continued.

life cycle at three of our study sites, while it was univoltine at the two southernmost sites ($<35.0^{\circ}$ N latitude). The other unexpected result was that the Karshi and Crete ecotypes were evidently reproductively active for a longer time period than was predicted for some of our sites based on their CDL. We will present two separate but related explanations for these discrepancies between the field experiments and the predictions made from the laboratory. These explanations should be treated as hypotheses that will need further testing, but which could have implications for the general understanding of seasonal adaptations in insects, as well as for how insects respond to environmental change.

The Fukang beetles used in our field experiment came from a field population that was released in 2001 near Lovelock in Nevada (40° N latitude). The Fukang

beetles had been in the field for at least 5 yr, at a site further south than the beetles' area of origin in Fukang, China (44° N latitude). The fact that the Lovelock population of the Fukang ecotype produced two generations at latitudes as far south as 35° N suggests that it had evolved new CDL in the United States, presumably shorter than the original population described in Bean et al. (2007a). Such shifts in daylength responses have been detected in other insects in response to environmental change, for example in the fall webworm (*Hyphantria cunea* Drury) after its range expansion in Japan (Gomi 2007), and in the pitcher plant mosquito (*Wyeomyia smithii* Coq.) in response to climate change (Bradshaw and Holzapfel 2001). Another observation supporting this hypothesis is that the Fukang populations in both Nevada and Colorado currently are reproductive for ≈ 10 d longer

than was observed during the first few years after the original release (D. W. Bean, T. Dudley, and D. Eberts, unpublished data). Reproduction later into the season can be explained by the evolution of a shorter CDL. Further studies are underway to reveal if this is the case. If so, this would suggest that the insects can adapt rapidly (within 5 yr) to new environmental conditions, leading to increased voltinism and spread southward. From an applied perspective, this implies that the Fukang population may be suitable for biological control at a wider range of latitudes in North America than previously believed (see e.g., Lewis et al. 2003, Bean et al. 2007a).

The other unexpected result was that the Karshi and Crete ecotypes produced generations outside of their predicted time-windows at some of our sites. For example, the Karshi beetles were reproductively active at least until September at latitudes around 34° N, although reproductive diapause should have been induced by mid-August based on the CDL measurements made in the laboratory at a constant 25°C. By early October, we found that ≈45–50% of the Karshi beetles were in reproductive diapause at the 2 southernmost sites, which indicates that the CDL had been met in the field. Past studies have shown that under field conditions there is usually a time-lag for diapause induction, from the time when the insects perceive the CDL until they enter diapause, taking ≈13 d in the Fukang ecotype (Bean et al. 2007a). Assuming a 13 d lag from perception of CDL to diapause induction, the Karshi ecotype would have perceived CDL during mid-September when day lengths are ≈12 h at the Santa Clara and Mojave sites. A field CDL of 12 h corresponds well to the CDL measured under a thermoperiod of 35°C (photophase) and 15°C (scotophase) in the laboratory that was 12 h 19 min (Fig. 1). In the Fukang ecotype, CDL was also shortened under a similar thermoperiod but only by ≈15 min (Bean et al. 2007a), indicating that CDL is more flexible in the southern Karshi ecotype than in the northern Fukang ecotype. While the critical daylength response of the Fukang ecotype seems relatively fixed and insensitive to temperature, the CDL of the Karshi ecotype may be modified by prevailing climate conditions. Overall, the results suggest that temperature effects on diapause induction differ among *Diorhabda* ecotypes and that this may explain some of our results.

One observation that seems to support the notion of southern *Diorhabda* ecotypes having a thermal modification of CDL comes from the comparison of the results from the Canadian River site in northern Texas (35.4° N) and the Santa Clara/Mojave sites (34.3° N/34.9° N) in southern California. In Texas, we found that 100% of the Karshi beetles were in diapause by early October, when at least 50% of the Karshi beetles were still reproductively active in California. Although the Texas site was located somewhat further north, the daylength by early October is similar at the three sites (~11.5 h). The main difference is that the climate is more continental (interior) in northern Texas with greater temperature variation between summer and winter. The temperature drops fairly rap-

idly in the fall and the foliage starts senescing by mid-October in northern Texas (E. N. Jones, unpublished data), whereas warm temperatures can extend host plant growing season for at least another month in southern California. Thus, the greater thermal modification of CDL in the southern ecotypes would allow those beetles to exhibit greater plasticity in diapause induction response, which could explain why the timing of diapause occurred later in California. Such a strategy for diapause induction may be adaptive in southern mild climate regions where the seasonal variations in temperature are not as extreme as they may be further north. In northern regions, such strategies could be risky if adult beetles produce offspring in response to prevailing warm conditions and if temperatures then drop during larval development. In the northern regions, it may therefore be adaptive to have a relatively fixed photoperiodic response for diapause induction. Further studies are suggested to compare the adaptive benefits of diapause strategies in northern and southern *Diorhabda* ecotypes at a wider range of latitudes and climate regimes.

The life cycle biology of the Turpan ecotype was bivoltine at the 2 southernmost sites, where the Fukang life cycle was univoltine. The results from the thermoperiod experiment revealed, however, that similar to the Fukang population, the Turpan ecotype also seems relatively insensitive to temperature for diapause induction. When tested under the fluctuating temperature regime, we found that 51% entered diapause at 14 h daylength (91% in diapause under 13 h 30 min daylength), which correspond to a CDL estimated at constant 25°C temperature. The Turpan ecotype had, however, a CDL (14 h 7 min) that was about 1 h shorter than the Fukang population described in Bean et al. (2007a). The Turpan population originates from latitude that is somewhat further south of the Fukang population (42.9° N versus 44.1° N). The main difference between the Turpan and the Fukang beetles is, however, that the Turpan beetles originate from an area that is lower in elevation compared with Fukang (Turpan located 70 m and Fukang 570 m above sea level) (DeLoach et al. 2003, Lewis et al. 2003). Previous studies have shown altitudinal variations in CDL, with a positive relationship between CDL and altitude (Bradshaw 1976). The shorter CDL in the Turpan ecotype may therefore partly be explained by a seasonal adaptation to lower elevation.

This study illustrates how seasonal adaptations to daylength in four ecotypes of a biological control insect affect voltinism in the introduced range. This was especially significant at the Mojave field site, where the northern Fukang ecotype produced only one generation whereas the southern Karshi ecotype was able to complete four generations before diapause. The plants still had green foliage at the time when Karshi beetles entered diapause. The results also highlight the effects of climate on insect voltinism. For example, the generation times of all ecotypes were shorter under the desert climate at Mojave River than under the coastal (milder) cli-

mate at Santa Clara River in California (Fig. 2). Both Karshi and Crete ecotypes were able to complete one additional generation at Mojave because of faster development, although the timing of reproductive diapause was similar at the two sites. Thus, although CDL may determine the timing of reproductive diapause in the field, climate conditions may determine how many generations the insects are able to complete before diapause is induced.

The Karshi ecotype seems especially promising for future biological control of saltcedar because it produced the greatest number of generations in the field experiment. This argument is based on the assumption that the more generations produced the more defoliation events and, thus, the more efficient control of saltcedar is expected. However, in a study by Milbrath et al. (2007), overwintering survival was higher for Crete than for Karshi in interior Texas. It was observed that the Karshi ecotype emerged from overwintering before saltcedar bud break in Texas, which is probably why the Karshi beetles had low survival (Milbrath et al. 2007). It is therefore possible that life-history traits other than diapause induction may need to be taken into account while matching biological control insects with environmental conditions. Further studies are therefore suggested to verify the factors affecting diapause termination and how beetle emergence is synchronized with *Tamarix* budburst in the spring. Biotic factors, such as the presence of predators (Lewis et al. 2003) and target *Tamarix* species (Dalin et al. 2009) are also important factors that may affect the establishment of *Diorhabda* beetles.

Many weed biological control programs have failed because of a mismatch between the agent and the environmental conditions of the release sites (Denoth et al. 2002). Future biological control programs should therefore consider investigating seasonal adaptations in the agent combined with field testing before the agent is to be released. Such investigations, like the ones described here, may facilitate the development of successful and cost effective biological control programs. Moreover, the release of biological control insects provides unique opportunities to study how insect life cycles may adjust to altered environmental conditions. When insects are exposed to novel climate or daylength conditions, insect life histories may change via plastic phenotypic responses or by genetic changes at the population level (evolution). In general, insect life cycle events determined by temperature should have a high level of phenotypic plasticity enabling rapid life cycle adjustments. Life cycle events determined by photoperiod, however, require evolution to achieve adaptive change (Yamanaka et al. 2008). Our study suggests that the relative importance of daylength and temperature for diapause induction vary among populations across latitude. Such information can be useful not only for matching biological control insects with environmental conditions, but also for understanding how insects respond to climate change in native regions.

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