

The Saltcedar Invasion: Analysis of Life Cycle Processes of a Biological Control Agent

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Abstract The exotic shrub known as saltcedar, or tamarisk (*Tamarix* spp.), has devastated western Riparian areas. A likely candidate as a device for biological control is the leafbeetle *Diorhabda elongata*, which tests have shown to be host-specific to saltcedar. Results from the release of these beetles in the field, however, indicate the failure of these beetles to survive in all states but Wyoming. The difference in the varying climates of the beetles' native area of Central China and the western U.S. is believed to be the primary cause. Motivated by their failure in southern states, such as Texas, with warmer climates, this study focused on the effects of high temperatures on the success of beetle reproduction or survival rate as larvae, pupa and beetles. Sample groups of *Diorhabda elongata* were subjected to constant temperatures of 30°C, 34°C, and 37°C, and also placed in various conditions of fluctuating day/night temperatures with averages at those values. Results showed that higher temperatures, while beneficial to larval survival rate, had an adverse effect on the percentage of pupa emerging as beetles. Fluctuating thermoperiods were mostly harmful for the survival rate of *D. elongata* as larvae; however, it is possible that these tests reaching 43°C was more harmful on the beetles than the fluctuation, since they thrived at one fluctuating test that reached a maximum of 43°C.

Introduction

Riparian areas are among the most important ecosystems in the Western U.S. for sustaining wildlife and biodiversity (Sanders and Edge 1998). They are fragile ecosystems, and have been adversely affected by pollution, land development and invasive animal and plant species (Allan and Flecker 1993). One such invasive plant species is the Tamarisk (*Tamarix* spp.), more commonly known as saltcedar. It is a shrub native to Europe and Asia, and is most abundant in Central Asia and the eastern Mediterranean (Baum 1978). With drastic population increase and widescale spreading, saltcedar is becoming a rising concern in the U.S. (Lesica and Miles 2001). In some areas, saltcedar replacement has even reached 100 percent (DiTomaso 1998).

Saltcedar affects riparian areas adversely in a number of ways (Lesica and Miles 2001). For one, it changes stream channels where saltcedar lines streams, increasing sedimentation and the overgrowth of sand and gravel bars, in some cases resulting in blocked channels (DiTomaso 1998). Also, it greatly reduces wildlife in the areas it invades; the number of bird species in saltcedar in the winter was under half that of native vegetation. Rodent populations, and insect numbers and biodiversity are all lower in saltcedar than in native vegetation (DeLoach et al. 1996, Sanders and Edge 1998). Additionally, saltcedar lowers the water table with its high water demand, excretes salt onto the soil, which, in turn, prevents certain species of plant from developing (Lesica and Miles 2001), and drastically increases the risk of forest fires due to its high flammability (DiTomaso 1998). Efforts are being made to find a viable, effective biological control, since they tend to reduce costs, can potentially cause little or no environmental harm, and target a specific species (Sprenger et al. 2001, McEvoy and Coombs 1999).

So far, the most successful biological control agent found has been the leafbeetle *Diorhabda elongata*, native to China and parts of Greece (Fornasari 1998). Studies have been run on other insects, but none have showed the level of host-specificity of the *Diorhabda* beetles (Fornasari 1997). After tests run on over 50 separate plant species found in riverine corridors of the Western U.S., *D. Elongata* has been found to feed exclusively on saltcedar (Fornasari 1997, Gould 1999). It has received USDA Animal and Plant Health Inspection Service (1999) approval, and has been introduced into 8 locations in six different states (Dudley et al. 2000).

Several problems, however, have arisen around the success of the beetles in the areas in which they have been introduced (Dudley et al. 2000). One problem relates to the natural process of insect induction into the state of diapause (Fornasari 1998). In this state the beetles go

through a type of hibernation, where no reproduction occurs (DeLoach et al. 1996, Leather et al. 1993). The two essential factors that influence the timing of diapause induction are temperature and photoperiod (Leather et al. 1993). The difference between temperature and photoperiod in problem areas of the U.S. versus the native habitat of *Diorhabda elongata* causes the beetles to go into diapause at an earlier time of year, adversely affecting population growth and causing decline to the point of, in some areas, complete failure (Fornasari 1997, Dudley et al. 2000). These beetles, in their native locations of the Old World, remain out of diapause for nearly the entire summer (Fornasari 1998). In the Southwestern U.S., induction into diapause occurs around mid-Summer, giving them a reproductive period of less than a month of (D. Bean: pers comm).

Temperature is also a factor in the induction of diapause of insects, as warmer temperatures tend to increase reproductive activity (Leather et al. 1993). Thus speculation arises as to why the warmer temperatures of the southern states, for example Texas, do not aid the beetles. The average temperature of a Texas summer day has been listed as approximately 37°C, and the *Diorhabda* beetles have not been tested at such a high temperature. This project is motivated by the question of whether it is possible that diapause is not the primary cause of the failure of these beetles in Southern states; namely, whether these beetles can simply not survive through the high temperatures of states such as Texas, New Mexico and Arizona.

The objective of this study is to discover how drastically high temperature conditions affect the ability of the *Diorhabda* beetles to maintain viable populations. Large groups of *Diorhabda elongata* will be subjected to a range of high summer temperatures experienced in areas of the Southwestern U.S. They will all be experiencing 14-hour simulated “day-periods”, which is an ideal photoperiod for *D. elongata* to remain out of diapause and survive as larvae and pupae. Thus, any observations made regarding diapause induction rate or survival rate will be a direct effect of temperature conditions.

The intent of this research was to determine whether these beetles have ability to survive through the temperatures of Texas, for example, regardless of the issue of photoperiod. The beetles were kept in incubators at a constant 37°C, a constant 34°C, and preliminary data at a constant 30°C was also available. The survival rate as larvae and the rate of pupating larvae emerging as adult beetles was recorded at all these temperatures.

However, these observations weren't only made for beetles set at constant temperatures. Previous studies have shown that neglecting variations in temperature when testing insects has a significant effect on results (Hagstrum and Milliken 1991, Brakefield and Massotta 1995). Therefore, beetles were also tested at fluctuating temperatures found in field release sites. Incubators that ranged from a minimum to a maximum over a uniform range that averaged out at one of the constant temperatures mentioned above not only ensured that the beetles were subjected to the high temperatures that are actually reached in the field, but also should reveal whether fluctuating temperatures at release sites harm populations that thrived in controlled laboratory environments.

My hypothesis was that the beetles subjected to fluctuating temperature conditions would have a significantly lower survival rate than beetles in incubators set at the constant temperature that the fluctuating incubator reached an average at. *D. elongata* subjected to extreme temperatures fluctuations where an intolerably high maximum was reached would experience drastically lower survival rate than those experiencing a smaller temperature range. Since preliminary data showed that the beetles had a relatively high survival rate at 30°C (D. Bean: pers.comm.), I estimate that survival rate will be significantly higher at 34°C, and then decrease at 37°C. If the beetles are subjected to temperatures above 37°C, a very small percentage of them will actually reach a healthy, reproductive adult stage.

Methods

A total of three incubators were available for the study; each could be set at different temperatures and photoperiods. The incubators were large enough to store up to six saltcedar plants and containers for the pupating *D. elongata*. All incubators were set at a photoperiod of 14 hours, with a 10-hour night, since this most accurately matches the photoperiod in mid-summer in the southern U.S. states being examined.

Due to the limited number of incubators available, the testing had to be divided up into two separate sections. A total of five separate tests were run; the first part of the experiment tested beetles at two separate temperature conditions, and the second part tested beetles at three separate temperature conditions. Data from the National Climatic Data Center (1998) stated that in the summer of 1998, in Dallas-Fort Worth, Texas, a minimum temperature of 37.8°C (100°F) was reached for 29 consecutive days. Using that value, to find a maximum threshold at which

beetles could survive, one incubator was set at a constant 37°C, while the temperature of another incubator fluctuated 6° during the course of a 24-hour day. The temperature was set at 31°C during the dark period, and 43°C during the middle 10 hours of the light period. The first two hours and last two hours of the light period were intermediate steps, where the incubator was set at the average, 37°C.

The second part of the testing required three incubators: one was set at a constant 34°C, and the other two were set at fluctuating thermoperiods with an average of 30°C. The fluctuating tests were assigned an average of 30°C because data from the National Climatic Data Center (1998) stated that from May-July of 1998, also in Dallas, a mean temperature of 28.67°C was recorded. This was rounded up to 30°C to match the preliminary data of beetles set at a constant 30°C. The reasoning behind the incubator set at a constant 34°C was to determine the maximum heat threshold of *Diorhabda elongata*, since no adult beetles emerged at 37°C. The test with more extreme fluctuations, termed '30°C fluctuating II', had a "dark-period" temperature of 17°C and 10-hour "day-period" temperature of 43°C, while the other, '30°C fluctuating I', ranged from 23°C to 37°C. It was not necessary to set up an incubator at a constant 30°C, since that preliminary data was already available from the USDA.

Each individual test began by adding beetle larvae to the incubators. Beetle larvae were kept together in large groups. For the first part of the experiment (at 37°C constant and 37°C fluctuating), each incubator had six potted saltcedar plants in it; for the second set of tests, each incubator had three plants, because not enough saltcedar plants were available to recreate the first set of tests. For the first part, each of the six plants in each incubator had either 20 or 25 larvae placed on it and were surrounded by a net to trap the beetles. For the second set of tests, 40 larvae were placed on each of the three saltcedar plants in each incubator. The plants were examined and watered every day, and as soon as the larvae were ready to pupate they were removed and placed in a container filled with sand. To detect a larva that was ready to pupate, the nets were examined and any larvae that have left the plant and lie on the net are in the pre-pupa stage (Gould 1999, Bean: pers. comm.). No container with sand held more than 25 pupa, and pre-pupating larvae discovered on different days were placed in separate containers. All containers were placed in the same incubator the potted plants were in. Any *D. elongata* that

reached a pre-pupating state was determined to have survived the larval stage; larval survival rate on every plant was recorded, as was the overall survival rate of all larvae in the incubator.

Once the pupa in the sand-filled containers emerged as adult beetles, they were removed from the sand-filled containers and were placed in new containers. For the first set of tests at 37°C constant and 37°C fluctuating, this was unnecessary, since no beetles survived the pupating stage. However, beetles did emerge from containers with the pupa subjected to a constant 34°C and in the 30°C fluctuating I incubator (23°C to 37°C). The new containers the adult beetles were placed in each had one male and one female beetle and an adequate food supply, continually stocked. Diapause was determined if no reproduction occurred within twenty days of the placement into these containers; this should have been ample time, since reproduction should occur within only ten days of placing two of these beetles together (Gould 1999). To conclude that the beetles were reproductive the plant clippings in the containers and the bottom and sides of the containers were closely examined for eggs.

To feed the leafbeetles, clippings of Saltcedar plants, which studies show are a preferred source of food for these beetles (DeLoach et al. 1996), were placed in the containers, and freshly rotated every 3 to 4 days.

A X^2 test was conducted on the overall survival rates, from a larval stage to an adult beetle, for beetles at all temperature conditions. Then, after eliminating the three tests where no beetles survived to an adult stage, another X^2 test was performed on the remaining two tests. Also, X^2 tests were conducted on beetle emergence rate data from a constant 30°C versus data from a constant 34°C, and beetle emergence rate data at 30°C versus 30°C fluctuating I (23°C to 37°C).

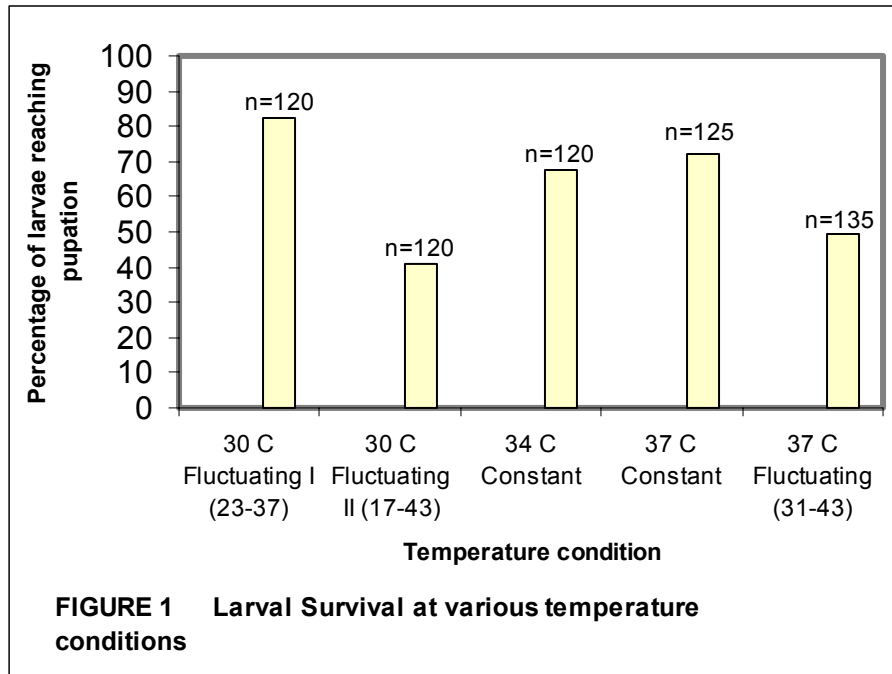
Results

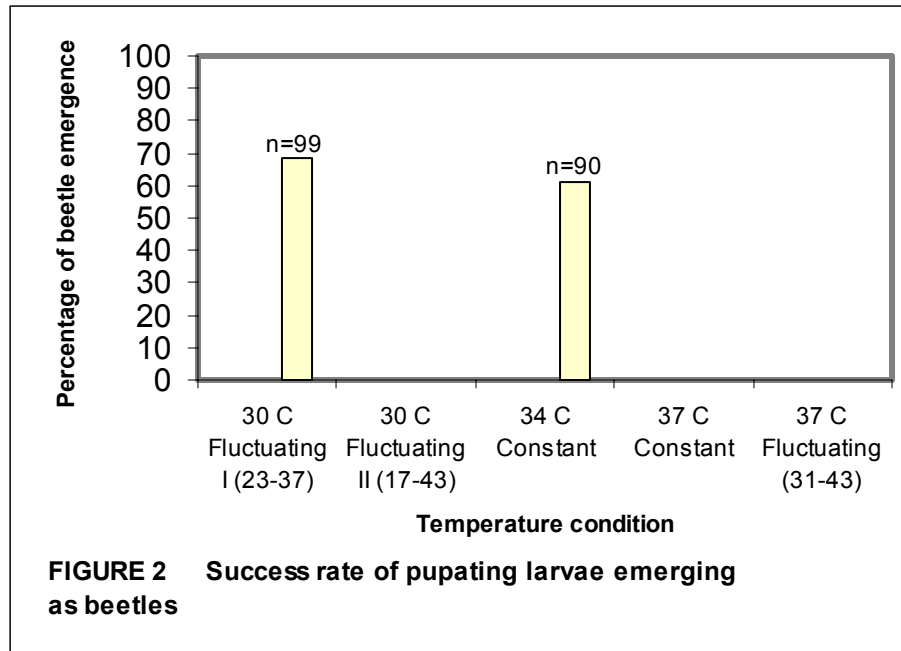
Survival rate for larvae at various temperature conditions was determined by comparing the number of beetles removed from their saltcedar plants and placed in sand when they were ready to pupate and the number of beetle originally added (Fig. 1). No replication was possible within each incubator, because the incubators had different numbers of plants and couldn't be compared with each other.

Results regarding the rate of pre-pupating beetles successfully entering and surviving the pupating stage and emerging as beetles could only be determined for *D. elongata* in two incubators: incubators set at a constant 34°C and a fluctuating-temperature incubator set at an

average of 30°C, ranging from 23°C to 37°C (Fig. 2). In all other tests, no beetles survived the pupating stage. Data regarding the rate of beetle emergence was already available for beetles subjected to a constant 30°C.

When determining the survival rate of larvae, the term “survival” refers to a beetle that has been removed from the netting of its host plant and placed in sand because it has reached a pre-pupating state. It does not imply that the larvae has begun pupation; in fact, all larvae, with the exception of three at a constant 37°, that did not emerge as beetles, never entered a pupating state, and were found dead at the surface of the sand.





A X^2 test was conducted on the overall survival rates, from a larval stage to an adult beetle, for beetles at all temperature conditions. With 4 as the degrees of freedom, a X^2 -value of 247.38 produced a P-value $<.0001$, and it was determined that temperature has a statistically significant effect on survival rate. Eliminating the three tests where no beetles survived to an adult stage, another X^2 was performed on the remaining two tests. Degrees of freedom was 1, the X^2 -value was 2.818, and the P-value was .093. This would imply that the differing temperature conditions from a constant 34°C to a fluctuating-temperature incubator set at an average of 30°C, ranging from 23°C to 37°C, did not produce a statistically significant change in survival rate.

Results from the X^2 test were conducted on beetle emergence rate data from a constant 30°C versus data from a constant 34°C revealed no significant difference due to temperature: a X^2 value of 1.758 at 1 degree of freedom gives a P-value of 0.1249. After conducting a X^2 test on beetle emergence rate data at 30°C versus 30°C fluctuating I (23°C to 37°C), a X^2 value of 0.317 at a degree of freedom of 1 resulted in a P-value of 0.6. According to these P-values, the differences in survival rate at the compared temperature conditions in these tests are not significant enough to draw the conclusion that the temperature differences affect beetle emergence rate.

Daily examination of the saltcedar clippings and containers indicated (due to the daily discovery of eggs) that all adult beetles were reproductive and had not entered diapause.

Discussion

The recorded data suggests that *Diorhabda elongata* are more sensitive to higher temperature as pupa than as larvae (Figs. I and II). While the 3°C temperature difference from a constant 34°C to a constant 37°C had no significant effect on survival rate, the effect it had on the percentage of beetle emergence out of the pupating state was drastic. While 66.1 % of all larvae that were added to sand-filled containers at 34°C emerged as adult beetles, no adults emerged in the constant 37°C incubator. This implies that the maximum constant temperature *Diorhabda* beetles can survive at lies between 34°C and 37°C.

The hypothesis that beetles subjected to fluctuating thermo-periods would have lower survival rates must be rejected. There was no significant difference between the beetle emergence rates at a constant 30°C versus the 30°C fluctuating I (23°C to 37°C) test. The data suggests that temperature fluctuation, on its own, has no significant effect on survival rate. Larval survival rate decreased by 41.7 % from the fluctuating test that reached a maximum of 37°C to the test that reached a maximum of 43°C. In the latter test, no adult beetles emerged. This suggests that the low survival of these beetles is an effect of the high temperatures reached during the light period, rather than the fluctuation itself.

All beetles that survived their given temperature conditions and reached adulthood were discovered to be reproductive, which supports the data from previous studies that increased temperature is beneficial in keeping *D. elongata* out of diapause. Additionally, this suggests that if the reason for the failure of *Diorhabda elongata* in southern states, such as Texas, is due to the beetles going into diapause prematurely, it is an effect of photoperiod in those areas, and not temperature. The data suggests that if temperature were the cause of the failure of these beetles to maintain a viable population in Texas and other southern states, it would be due to the temperatures being so high that the beetles couldn't survive through their larval stage and pupation, and not diapause induction.

The hypothesis that survival rate would increase significantly from a constant 30°C to a constant 34°C is questionable, since the χ^2 test conducted on the beetle emergence rates at those temperatures revealed no significant change in the percentage of pupae that reach adulthood. The problem with this analysis, though, was the lack of data available for *D. elongata* at 30°C;

data was only available for beetle emergence rates, and not larval survival or the reproductive state of the beetles. Thus, comparisons could only be made for beetle emergence rates, and not overall survival rates as in other analyses.

Another observation of note is the difference in overall survival rate between the mild and extreme fluctuating tests conducted at an average of 30°C. The data indicates that if *Diorhabda elongata* are subjected to 43°C on any regular basis they will not reach an adult state (Fig. 2). Even for the beetles' survival as larvae, which appears to be less sensitive to high temperatures, the two tests that reached maximums of 43°C had the lowest survival rates (Fig. 1).

The lack of replication between tests made it difficult to statistically analyze the tests in as much detail as the experiment warranted. For one, the two tests conducted first used six potted saltcedar plants in each incubator, while the other three tests used three potted saltcedar plants per incubator. Also, for the first two tests, the number of larvae placed on each plant was not constant; thus, replication was not possible within each incubator. Results would have been more statistically sound if all five incubators had housed the same number of plants and the same number of larvae was initially placed on each plant. Under those methods, I would have been able to calculate the survival rate of larvae on each individual plant and calculated the mean of those values as the final survival rate for that test. With this replication, I could have also conducted χ^2 tests comparing the survival rates on all plants in each incubator to ensure that there wasn't excessive variation from plant to plant.

Given the limited time and resources available in which to perform this experiment, it would have probably been wiser to either focus on finding a maximum temperature at which the beetles could survive, or determining the effect of a fluctuating thermoperiod, rather than both. A credible study that focused on finding the highest constant temperature *D. elongata* could survive at would involve setting up six incubators, ranging from 32°C to 37°C at 1°C interval. To determine the effect of temperature fluctuations, one could set up a study where all tests have the same average temperature and only vary in range between night and day temperatures.

Recorded data shows that a relatively high amount of beetles reach a reproductive adult state at a constant 34°C (Fig. 2), and the χ^2 test comparing the preliminary data at a constant 30°C to the 30°C fluctuating I (23°C to 37°C) test shows that fluctuating thermoperiod does not have a significant effect on beetle emergence rate. It is possible that these two observations would imply that, excluding all factor other than temperature, *Diorhabda elongata* would survive in

areas with an average of 34°C. Texas, where these beetles had their worst failure of the 8 release sites in the Southwestern U.S. (Dudley et al. 2000), had an average temperature of 28.67°C, which was a record high, in the summer of 1998 (NCDC 1998), 5.33°C lower than the estimated heat threshold. This would most likely indicate that a factor other than temperature is causing the failure of *D. elongata* to maintain viable populations in Southwestern U.S. states. Due to the difference in latitude, and thus day-lengths, between the native habitat of *Diorhabda elongata* versus the Southwestern U.S., it is most plausible that the original hypothesis stated by Dudley et al. (2000) and Fornasari (1998) regarding the premature diapause induction caused by shorter summer photoperiods being the primary cause of their failure to survive is correct.

The U.S. Department of Agriculture has recently received large shipments of *D. elongata* from riparian areas in Greece (D. Bean: pers. comm.). Because the climate and latitude of this area is more similar to that of the Southwestern U.S. than the native habitats of *Diorhabda* beetles in China (D. Bean: pers. comm.), it is hoped that these beetles will experience more success than the specimen of *Diorhabda elongata* tested in this study.

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