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Monarch Butterfly (Danaus Plexippus) Conservation in Central Mexico: Survival Analysis in Asclepias Physocarpa Crops

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Abstract

Habitat loss and pesticide use are the main threats for *Danaus plexippus*. Valle de Bravo is in Central Mexico, has a population density from two to five million butterflies per km². However, wild milkweed populations have declined and local producers grow *Asclepias physocarpa* applying pesticides. In this study, monarch butterfly survival was evaluated in *A. physocarpa* crops with two treatments: with carbamate-type pesticides and without pesticides. In addition, the relationships of population parameters of *D. plexippus* with environmental parameters and characteristics of the host plant, were analyzed. The use of pesticides reduces the survival of *D. plexippus* by up to 50%. The most vulnerable stages are larva I and larva III. The environmental temperature of 18° C to 19° C and radiation of 350 lum/ft² to 650 lum/ft² favor the laying of eggs. The monarch butterfly oviposited 80% of its eggs in plants of 100 cm ± 45.35 cm, with hatching success of 80%. Implications for insect conservation: our study suggests conservation policies that encourage the cultivation of native milkweed and eliminate or reduce the pesticide use and divulgate scientific information to local people.

Introduction

The insect populations are drastically decreasing, the most affected taxa is Lepidoptera, 53% of its species are in danger (Sánchez and Wyckhuys 2019; Wilcox et al. 2019). In Mexico, the monarch butterfly *Danaus plexippus*, has migratory and resident populations, is under special protection (SEMARNAT 2010). The main threats to its conservation are logging, urbanization and predation of the adult stage (Hunt and Tongen 2017; Flores et al. 2020). However, according to studies in the United States and Canada, the main factors that affect the survival rate in the different stages of the monarch butterfly are the use of pesticides (Hartzler 2010; Pleasants and Oberhauser 2013; Zalucki et al. 2016; Krishnan et al. 2020; Krueger et al. 2021), and fragmentation and habitat loss (Thogmartin et al. 2017; Zaya et al. 2017).

In Mexico, the Monarch Butterfly Biosphere Reserve (MBBR) in Central Mexico includes Valle de Bravo and other municipalities, where there are from two to five million butterflies/ km², this is favored by the fir forest and milkweed crops (López et al. 2011). In Valle de Bravo, it has been observed that wild milkweed has decreased due to extraction for sale as ornamental flower. Some inhabitants have cultivated *Asclepias physocarpa* native from Africa for commercial use (Naturalista 2020). In the cultivation of this species the local producers apply different doses of carbamate pesticides (Furadan® and Lannate®; Moreira et al. 2015) to eliminate mainly monarch butterfly eggs and larvae.

Previous studies report low survival rates during the life cycle of the monarch butterfly due to the use of pesticides (organophosphates and neonicotinoids; Zalucki et al. 2016; Krishnan et al. 2020; Krueger et al. 2021), and by the decrease of host plants (*Asclepias* spp., *Asclepias syriaca*) due to the cultivation of corn and soybeans (Hartzler 2010; Pleasants and Oberhauser 2013; Thogmartin et al. 2017; Zaya et al. 2017). Also, the cultivation of transgenic corn with pesticide toxins causes low survival in monarch butterfly larvae due to the natural dispersal of transgenic pollen to host plants of *A. syriaca* (Stanley et al. 2001). In Mexico, the impact level of pesticides on survival in immature stages of the monarch butterfly

has not been documented. Therefore, the objective of this study was to compare the survival of *D. plexippus* of immature stages (egg, larva, and chrysalis) in *Asclepias physocarpa* crops with pesticide application and without pesticide. Population parameters of *D. plexippus* and their relationship with characteristics of the host plant *A. physocarpa* and environmental parameters were also evaluated to generate conservation actions and increase survival percentage.

Materials And Methods

Study area

Valle de Bravo is located in Central Mexico to the southwest of the Trans-Mexican Volcanic Belt, with average altitude of 2,445 meters above sea level. It is located between 19° 17' 27"N and 19° 4' 43" N, and between 100° 15' 15"W and 99° 57' 33" W (Fig. 1). The average annual temperature is 13.7° C and annual precipitation ranges from 900 to 1,300 millimeters. The dominant vegetation is fir forest, pine forest and mountain cloud forest (López et al. 2011).

Commercial milkweed (*Asclepia physocarpa*) were grown at two sites. At each site, two plots of 100m² each (10 x 10m²) were cultivated. One plot at each site was treated with pesticide in the traditional way (S1P; 19° 13' 55”N 100° 04' 45” W, S2P; 19° 14' 27”N and 100° 05' 15” W) and the second plots were without pesticide (S1NP; 19° 14' 28 ”N and 100° 05' 14” W, S2NP; 19° 13' 56”N and 100° 04' 44” W). The plots with pesticide had different doses of carbamate (Furadan® and Lannate®), by medium pressure spraying; S2P had an application of 30gr/ 20l, seven days after sowing, while S1P had four applications each of 30gr / 20l every seven days. Each plot was divided into four 2.5m x 10m quadrants. In each quadrant 25 plants were measured and monitored; recording in each one the number of eggs, larvae or pupae. Each plant was labeled with the following data: plant number, observation date, plot and treatment, plants with eggs were marked and laveled. Upon hatching and passing to the second larval stage, each larva was marked with a unique code (vinyl-acrylic paint, organic Vinimex® COMEX® orange color, 1: 1 ratio with water; Mikery et al. 2013) on the thorax and abdomen to monitor their survival from egg to chrysalis. Larval stages were identified considering the head size and filament length in the thorax and abdomen (Yeargan and Allard 2005;). In each plot, the plants with presence of eggs, larvae, and pupae were monitored every four days for 30 days. The relationship of plant characteristics with oviposition and hatching success (larvae and butterflies) and with larval survival was analyzed De la Pava and Sepúlveda 2012). Two data-loggers were installed in each plot without pesticide to measure the temperature and solar radiation every two hours. It was analyzed whether these parameters are related to oviposition, hatching success (larvae and butterflies) and survival of monarch butterfly larvae. Survival of the larvae was evaluated by stage and treatment through cohort-type life tables and survival curves (Romero and Cortina 2007).

Survival curves were obtained to determine vulnerability by stage. Normality tests were performed on each variable analyzed. Two student's t tests were performed, one to compare survival between plots and the other between treatments. An ANOVA analysis was performed to identify the significant differences in

survival between treatments and stages of *D. plexippus*. The relationship between environmental parameters (temperature and solar radiation) and oviposition, hatching success (larvae and butterflies) and survival of monarch butterfly larvae were evaluated using Pearson's correlation analysis. The relationship between plant characteristics (height, number of leaves and number of inflorescences) with oviposition, hatching success (larvae and butterflies) and survival of monarch butterfly larvae were evaluated using Pearson's correlation analysis. It was analyzed if there is a pattern between the average height of the plants and oviposition, hatching success and larval survival.

Results

706 monarch butterfly egg layings were identified in 400 host plants of *A. physocarpa*. The plots with pesticide had fewer eggs (196) than the plots without pesticide (510). Survival has values from zero to one, where the eggs had the highest value and in the following stages (larva I, larva III, larva V, pupa) survival decreased (Table 1).

Table 1
Survival rates by stage and treatments (Pesticide and notpesticide) of *Danaus plexippus* in Central Mexico.

Treatment	Pesticide		No pesticide	
	Site 1	Site 2	Site 1	Site 2
Eggs	1	1	1	1
Larvae I	0.244	0.455	0.597	0.750
Larvae III	0.163	0.251	0.508	0.711
Larvae V	0	0.095	0.367	0.693
Chrysalis	0	0.088	0.274	0.306

Exist significant differences in monarch butterfly survival between stages by treatment: with pesticide (S1P + S2P; $F = 12.81$, d.f.= 4.36; $P < 0.0002$) and without pesticide (S1NP + S2NP; $F = 10.49$, d.f.= 4,36; $P < 0.0000$). In the treatment without pesticide (S1NP and S2NP) the average survival was higher (0.526 ± 0.190) than in the treatment with pesticide (S1P and S2P; 0.162 ± 0.153, $t = 4.208$; d.f.= 14; $P < 0.0004$). The survival curve of the treatments with pesticide (S1P + S2P) and without pesticide (S1NP + S2NP), show that the most vulnerable stages are larva I and larva III in the pesticide treatments. The survival curve of pesticide treatment is type III in which survival in the first stages decreases drastically; while in treatment without pesticide it is type II in which constant survival is observed throughout the larval phase (Fig. 2).

The sites did not influence the survival of monarch butterfly, no significant differences were found ($t = -1.20823$; d.f.= 14; $P < 0.2469$). In the site not pesticide S1NP, a dose of 30 gr/ 20l was applied, and

surviving larvae in fifth stage with incomplete antennae and poor segmentation development were observed.

The temperature (r^2= 85.26%) and solar radiation (r^2= 88.14%) were correlated with the oviposition of monarch butterfly in the plots without pesticides. No relationship was found between temperature and solar radiation with the successful hatching of larvae, survival of larvae, or with the success of hatching of *D. plexippus*. Oviposition initiation was recorded from 16°C with radiation of 350 lum / ft². Between 18° to 19°C and solar radiation from 630 lum/ ft² to 650 lum/ ft², there was greater oviposition.

The height of the plants in the plots not pesticide (S1NP and S2NP), was correlated with oviposition (r^2= 40.86%), larval hatching success (r^2= 48.49%), larval survival (r^2= 45.50%) and the hatching success of butterflies (r^2 = 37.32%). On the other hand, the number of leaves and the number of inflorescences were not related to any parameter evaluated in the monarch butterfly. In plots not pesticides, female monarchs laid 80% of their eggs in plants of 100 cm ± 45.35 cm, the hatching success of larvae in plants of 100 cm ± 62 cm was 90%. Stage III larvae moved to 65 cm and 66 cm plants in which 76% survived. During the last larval stage, the V larvae moved to 69 cm plants in which they formed the chrysalis and the hatching success of butterflies was 80%.

Discusion

This is the first experimental study where the negative effect of pesticides on the survival of larval stages of *D. plexippus* is quantified. The treatments without pesticide had more eggs (510) than the treatments with pesticide (196), this is probably due to the chemosensory tarsal receptors that the butterfly have in its appendages, since they detect the chemical composition on the surface of the leaves. Monophagous species such as *D. plexippus* that feed on a family of plants respond to food stimuli such as sugars or deterrents such as pesticides in a very specific way, modifying their oviposition behavior (Honda 1995; Olaya-Arenas et al. 2020). Furthermore, by means of epidermal pheromones from the appendages or in larvae, females can mark their host plants to indicate optimal oviposition sites to other females of the same species (Aluja and Díaz-Fleischer 2006; Ramos et al. 2020).

In the treatment without pesticide, the survival rate was higher than in the treatment with pesticide (S1NP 51.23% and S2NP 54.59%); which coincides with butterflies previous studies, proving that *D. plexippus* larvae that fed on milkweed leaves with pesticide had a decrease in survival between 20% and 40%, the same has been documented in other butterflies species as *Pieris brassicae* and *Polyommatus icarus* (Krischik et al. 2015; Belsky and Joshi 2018; Crone et al. 2019; Pelton et al. 2019). Pyrethroid, organophosphate and neonicotinoid pesticides used in agriculture have been reported to affect insects, including monarch butterfly larvae (James 2019; Krishnan et al. 2020; Halsch et al. 2020).

The survival curve with pesticide treatment is type III, in which survival in the first stages decreases dramatically due to the pesticide, which intoxicates the insects that consume it. Two studies carried out in the United States show a similar behavior of the type III curve caused by indirect exposure to

pesticides. During the first three stages, the monarch butterfly larvae consume the greatest amount of leaves to increase their size by between 30% and 40% (Fisher et al. 2020); this would explain the behavior of the curve and the vulnerability of larvae I and III exposed to pesticides. The survival curve of the treatment without pesticide was type II, where mortality is constant in all stages and can occur due to natural factors such as predation or infertile eggs (Fig. 2; Zalucki et al. 2001; Oberhauser et al. 2001).

In Valle de Bravo the pesticides used in milkweed (*A. physocarpa*) crops are Lannate® (methomyl chemical group) and Furadan® (carbofuran chemical group), both from the group of carbamate insecticides (FAO 2001; Moreira et al. 2015). Carbamates are organic compounds derived from carbamic acid (NH_2COOH) that inhibit the enzyme acetylcholinesterase generating an overstimulation of the nervous system that contains acetylcholine, causing damage to larval development, metabolic and enzymatic alterations in insects (DiBartolomeis et al. 2019; Barreto et al. 2020). This causes teratogenesis, mutagenesis, carcinogenesis and death in high doses (> 5 mg/ kg), which would explain the low survival rates in pesticide treatment (WHO 2009; Moreira et al. 2015; Thogmartin et al. 2017). The toxicity of carbamate pesticides varies according to the dose and type of absorption (Moreira et al. 2015). They are also classified by the World Health Organization (WHO), by the Food and Agriculture Organization of the United Nations (FAO) and by the Environmental Protection Agency (EPA) as highly toxic pesticides because they eliminate 50% of the insect population in the sites where they are applied (FAO 2001; WHO 2009).

The dose of carbamate pesticide approved by WHO is from 0.5 to 1 mg/kg, three applications during a growing season, with interval of 30 to 45 days between applications (WHO 2009). However, in the study area the applied dose was higher than this. In the plot with pesticide S1P the survival rate was lower than in S2P, because in S1P the pesticide application was carried out four times, every seven days; while in S2P it was an application seven days after sowing. The surviving larvae presented malformations such as: incomplete development of antennae and segments; this could be caused by carbamates pesticides in permissible doses of 1 mg / l or < 1 mg / l (Bhattacharyya et al. 2015). The survival rate in the treatment without pesticide was modified due to biological interactions that were observed in the site, such as predation by bed bugs *Padaeus trivittatus* that feed on monarch butterfly larvae (Haan and Landis 2019). In addition, due to competition with the species *Euchaetes egle* and *Aphis nerii* which, during the larval stage, also specifically consumes milkweed foliage (Haan and Landis 2019; Herman et al. 2019). Most pesticides, including carbamates, also affect other species. In addition, these pesticides cause environmental pollution, mainly through seepage to groundwater, seepage of waste or accidental spills. Pesticide remains are dispersed in the environment and affect more elements of the ecosystem that over time become a danger to human public health (Varona et al. 2009; Del Puerto et al. 2014; SEMARNAT and CONANP 2018; James 2019).

In Valle de Bravo the use of pesticides has been common for ten years for the cultivation of flowers and other products that sustain part of the municipality's economy, between them flower crops such as milkweed (*A. physocarpa*), sunflowers (*Helianthus annuus*), bird of paradise (*Strelitzia reginae*), white house (*Lilium candidum*), pumpkin flower (*Cucurbita pepo*), fruit and vegetable crops such as avocado

(*Persea americana*), lettuce (*Lactuca sativa*), coriander (*Coriandrum sativum*) radish (*Rhaphanus sativus*) and seeds such as corn (*Zea mays*), beans (*Phaseolus vulgaris*) and broad bean (*Vicia faba*; Pérez and Pérez 2004; Naturalista 2020).

This constant and excessive use of pesticides has relation with problems for human health. Human exposure to these substances can be direct (people in industries that manufacture them or farmers that apply them) or indirect (consumers or people close to crops where they are applied). Pesticides have chronic effects on human health according to the dose and type of exposure: a) acute toxicity, caused by the ingestion of any pesticide on one occasion in doses of DL ; b) dermal toxicity, due to skin contact with the pesticide in doses above the LD ; c) inhalation toxicity, by breathing in any place the pesticide by nebulization, spraying and atomization (Del Puerto et al. 2014; Avinash et al. 2015; Marrero et al. 2017; SEMARNAT and CONANP 2018).

In Mexico, there are studies on environmental parameters such as temperature, precipitation and humidity, that have determined the optimal conditions in hibernation sanctuaries and explained the influence of these fluctuations on the survival of *D. plexippus* (Mejia and Arellano 1992; Larsen and Lee 1994; Anderson and Brower 1996). However, information is lacking on environmental parameters that are related to oviposition such as temperature and solar radiation. The results of this study suggests that the environmental parameters of temperature and solar radiation are related to the oviposition of the monarch butterfly.

According to other studies, the optimal temperature range for oviposition and development of *D. plexippus* is between 27° C and 29°C, temperatures below 20°C decrease the hatching of eggs. In the study area the temperature was lower than the optimum reported; however, the latitudinal adaptation of the monarch butterfly to variations in temperature, precipitation and humidity in the tropics has been suggested (York and Oberhauser 2002). There is no information on the relationship between solar radiation and oviposition or larval development. However, as there is a relationship between both variables, it is evident that the optimal solar radiation for oviposition is between 630 lum/ft² and 650 lum/ft² (Fig. 5). Furthermore, from 16°C with a radiation of 350 lum/ ft², the monarch butterfly begins to oviposit, which suggests a tolerance to lower temperature ranges (York and Oberhauser 2002; Batalden et al. 2007).

The monarch butterfly laid 80% of its eggs, in plants with an average height of 100 cm (+ 43.5 cm), in which the hatching success was 90%. In a study in Australia *Asclepias physocarpa* with a height of 54 to 100 cm had more eggs, due to the high quantity of cardiac glycosides, which reduces interactions between species due to competition or predation; in addition, in taller plants it decreases the moisture concentrated in the soil, preventing freezing that causes slow maturation and development, and low survival rates (Zalucki and Kitching 2009; Pitman et al. 2018; Haan and Landis 2019). This also explains the movement of the III larvae that were found in plants between 120 and 150 cm, to plants of 65 cm to 66 cm. The V larvae also moved to 69 cm plants in which they formed the chrysalis and increased their butterfly hatching.

Management proposals for *Danaus plexippus* in Central Mexico

The conservation of *D. plexippus* depends mainly on two factors: 1) recovering the places of reproduction and hibernation and 2) reducing the use of pesticides. This study suggests immediate intervention to restrict or eliminate the use of pesticides in Central Mexico, specifically in the monarch butterfly sanctuaries and nearby areas.

1. Policies. The North American Plan for the Conservation of the Monarch Butterfly (NAPCMB) and the Commission for Environmental Cooperation (CEC) have generated proposals between Canada, U.S.A. and Mexico to conserve the monarch butterfly and its migratory phenomenon. In Mexico, this plan focuses on actions to conserve the forest in hibernation sites, avoiding logging, changes in land use, and fires (SEMARNAT and CONANP 2018). However, this study shows the importance of maintaining and increasing pesticide-free breeding sites to increase survival rates in resident and migratory populations of monarch butterflies.

2) *Sites with native milkweed.* It is important to motivate farmers and landowners to implement actions to protect, improve and restore the breeding sites of the monarch butterfly. Increasing and maintaining the breeding sites should be a priority, sowing *Asclepias* spp., in farmland, agricultural areas, and urban gardens free of pesticides; also propagate native milkweed plants (Thogmartin et al. 2017).

3) *Pesticide reduction.* The use of pesticides in sanctuaries and places near milkweed sites should be eliminated or reduced. According to this study and others, it is necessary to stop the use of highly toxic pesticides such as neonicotinoids, pyrethroids, glyphosates, and carbamates, because even in low or controlled doses they reduce the survival of the monarch butterfly. Reducing dependence on pesticides implies using biological control or organic pesticides, minimizing risks to non-target organisms, in addition to risks to human health humana (SEMARNAT and CONANP 2018; Crone et al. 2019; James 2019).

4) *Environmental education*. Scientific information about the biology and conservation of the resident and migratory monarch butterfly in Mexico should be disclosed. In addition to the risks of use pesticides for the environment and human health. A management plan should be generated for the breeding sites including the management of native milkweed without highly toxic pesticides and alternatives to substitute them (Epstein y Bassein 2003; SEMARNAT and CONANP 2018; Crone et al. 2019). The use of carbamate pesticides (Furadan® and Lannate®) in *A. physocarpa* crops reduces the survival of *D. plexippus* up to 50%, the most vulnerable stages are larva I and larva III. The highest egg laying occurs between 18° to 19°C and between 350 lum/ft² to 650 lum/ft². Females of *D. plexippus* oviposited 80% of their eggs in plants of 100 cm ± 45.35 cm, with a hatching success of 80%. The larvae III moved to 65 cm and 66 cm plants and increased their survival to 77%, while the V larvae moved to 69 cm plants in which they pupated and increased butterflies hatching to 80%. The previous analysis suggests immediate intervention to eliminate or reduce the use of pesticides in the breeding sites of *D. plexippus*. Policies need to be added to the North American Plan for the Conservation of the Monarch Butterfly: increase and maintain milkweed sites, replace invasive species such as *A. physocarpa* with native species, using

techniques that favor the conservation of the monarch butterfly and implement environmental education that includes community science and management plans for breeding sites.

Declarations

Contribution of authors

OM-V: Project design, analysis, and paper writing. S.F.B-P: Data collection, analysis and writing paper. M.M Z-G: Analysis and paper writing

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Figures

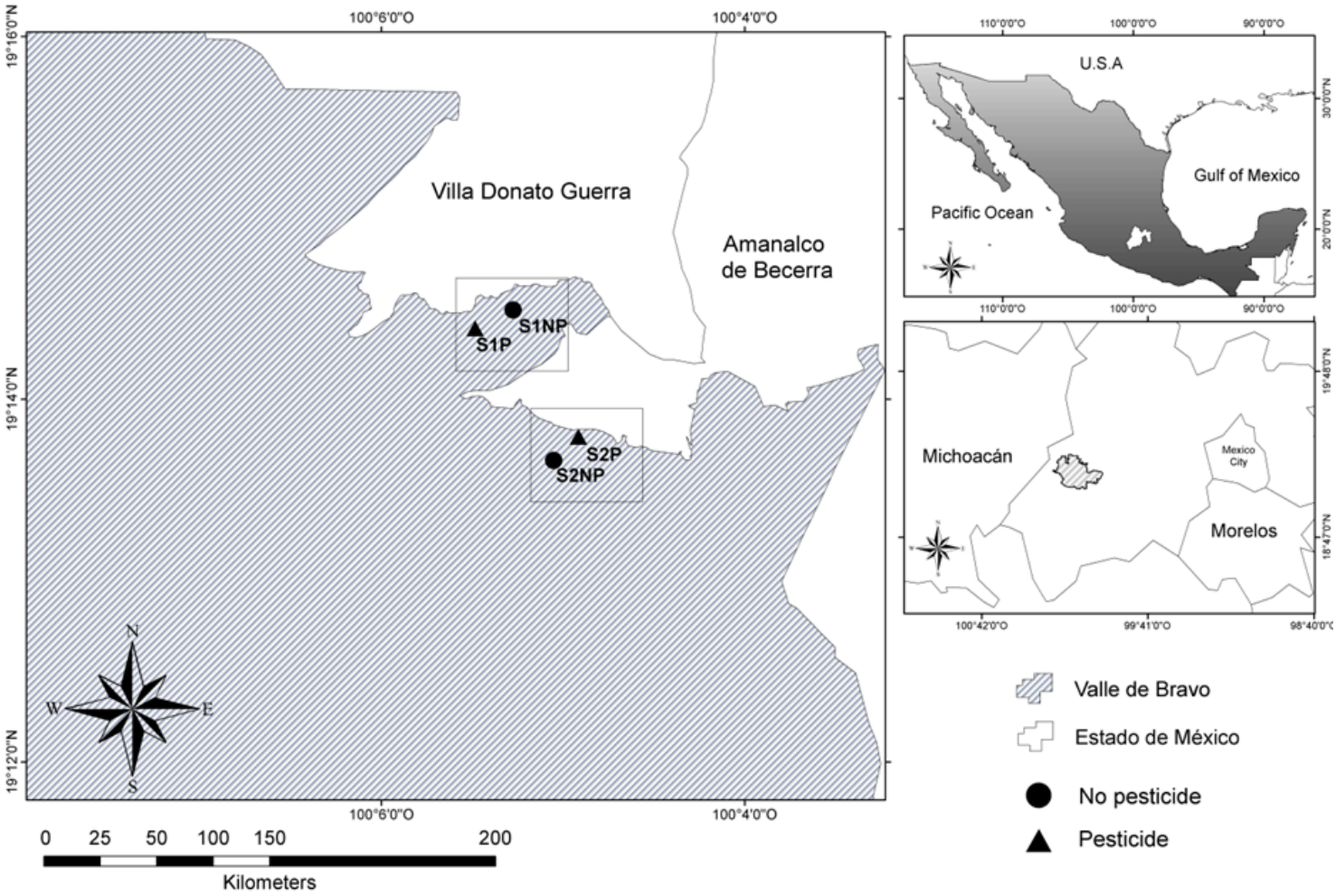


Figure 1

Valle de Bravo, sampling sites with pesticide (S1P and S2P) and without pesticide (S1NP and S2NP) for the analysis and evaluation of the survival of the monarch butterfly (*Danaus plexippus*).

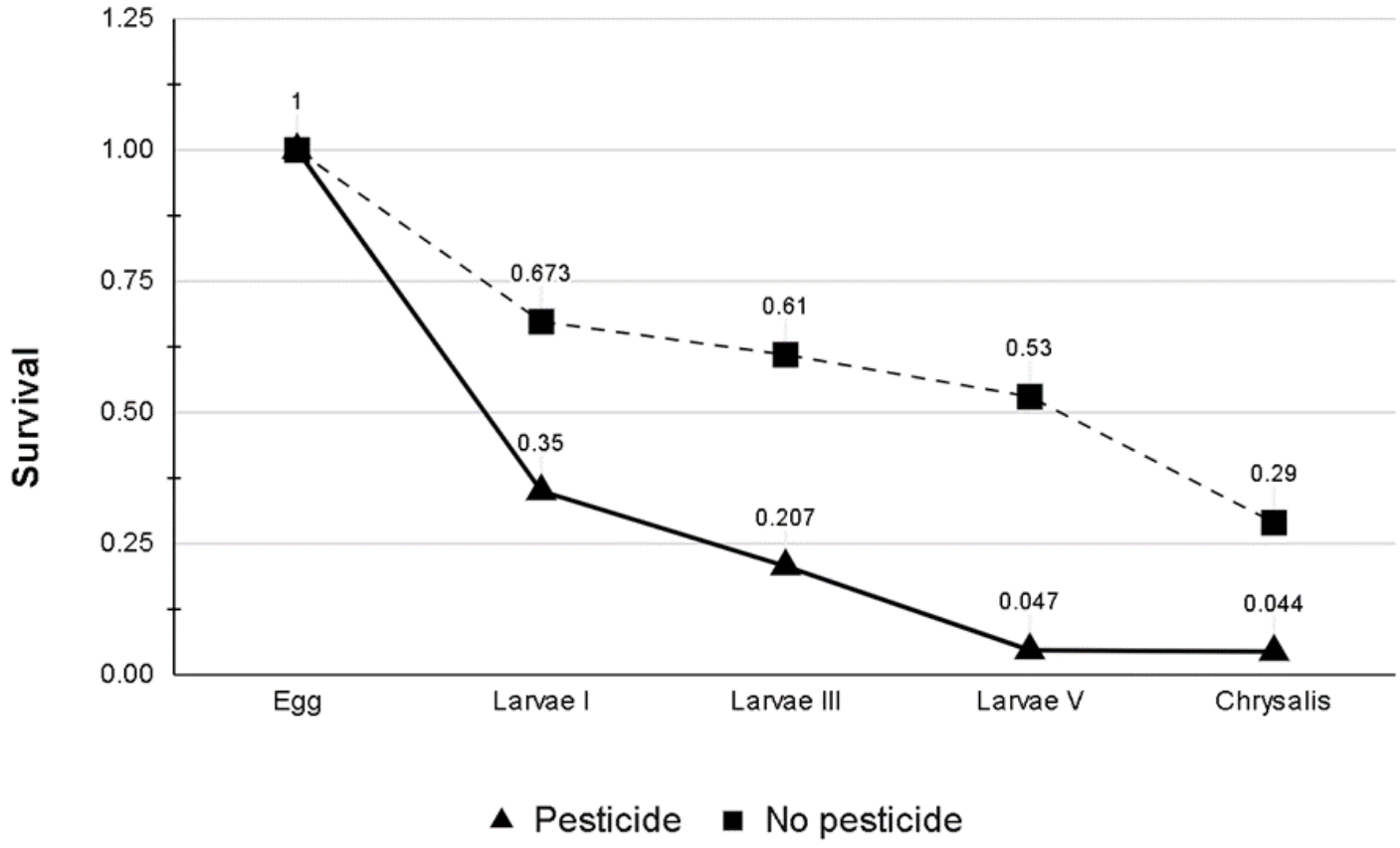


Figure 2

Survival curves of treatments with pesticide (S1P + S2P) and Not pesticide (S1NP + S2NP) by stage of *Danaus plexippus* in Central Mexico.