Survival of Seasonal Flooding in the Amazon by the Terrestrial Insect Conotrachelus dubiae *O'Brien & Couturier (Coleoptera: Curculionidae), a Pest of* the Camu-Camu Plant, Myrciaria dubia *(Myrtaceae)*

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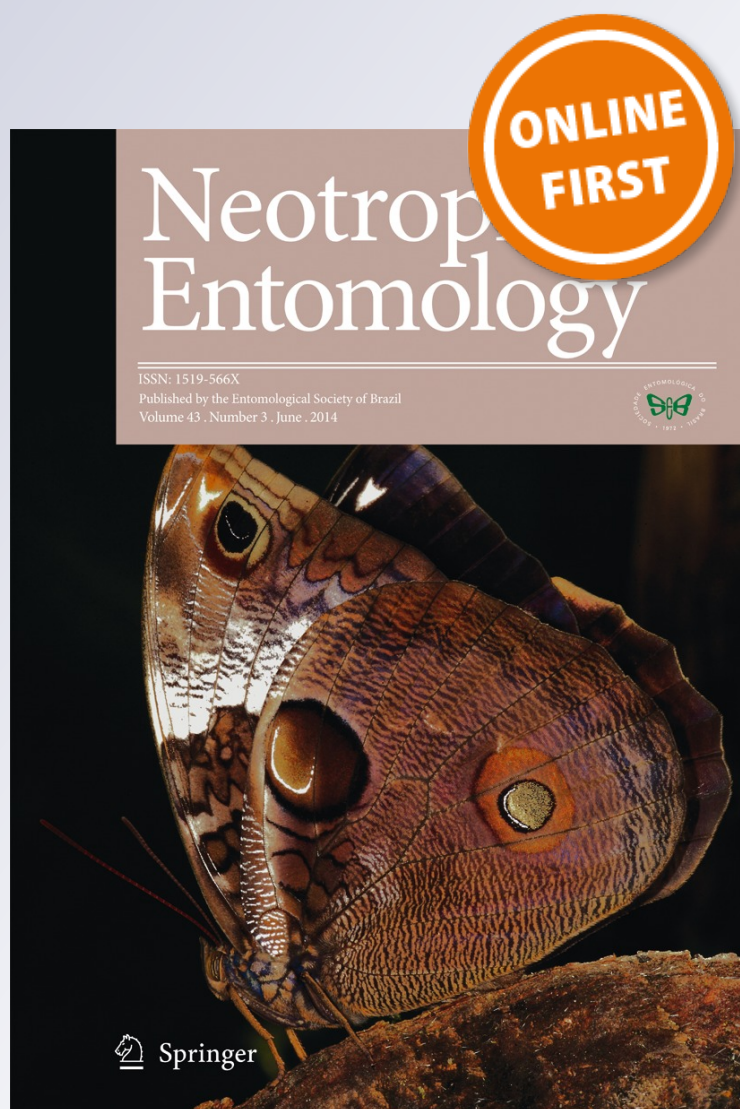
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Abstract

The weevil *Conotrachelus dubiae* O'Brien & Couturier (Coleoptera: Curculionidae) is a pest of an economically important Amazonian fruit tree *Myrciaria dubia* (Myrtaceae). This tree grows in seasonally flooded environments, and how weevil larvae survive flooding has not been studied. From December 2004 to May 2009, five experiments were conducted in natural conditions and in the laboratory, with the aim of understanding the mechanisms that allow the survival of *C. dubiae* larvae in seasonal floods in Amazonia. The larvae of *C. dubiae* were kept under water for over 93 days. Older instars exposed to periodic circulation of water survived better than younger instars in addition to all larvae that were kept continuously under uncirculated water. Individuals that were collected from plots of *M. dubia* located in flooded soils and non-flooded soils did not exhibit statistically significant differences in their levels of survival indicating that the variation in survival of flooding events is due to phenotypic plasticity of the species and not to local adaptation by the populations in different environments. We speculate that larvae can survive floods without major physiological changes as larvae appear to obtain oxygen from water by cutaneous diffusion, assisted by caudal movements.

Introduction

Terrestrial invertebrates inhabiting flooded systems require special survival strategies (Adis 1997, Adis & Junk 2002, Plum 2005). The development of these strategies is determined by the type of flooding, which can vary by the frequency, depth, and duration of flooding events in the course of a year (Junk *et al* 1989, Plum 2005). *Myrciaria dubia* (Myrtaceae), known locally as camu-camu is native to the blackwater floodplain forests (*tahuampas*) of the Amazon basin. It can form dense, almost monospecific stands covering up to 60 ha. These plants remain submerged in water up to 12 m deep for a period of 5–6 months during the year (Peters & Vásquez 1987). When the water level begins to subside, the plants flush new leaves, after which they begin flowering. It takes

on average 162 days from leaf flushing to fruiting period (Inga *et al* 2001). Fruiting in flooded soils occurs once a year, coinciding with the start of the flood cycle, while in non-flooded soils, fruit production is continuous but includes two peaks of maximum production during the calendar year (Maués & Couturier 2002, Rodrigues *et al* 2001).

Conotrachelus dubiae O'Brien & Couturier (Coleoptera: Curculionidae) is a small beetle (0.6 cm in length) and has been described as an important pest of *M. dubia* (O'Brien & Couturier 1995). The females lay their eggs on the fruits, and the larvae feed on the pulp and seeds and abandon the fruit after they develop, falling to the ground and burying themselves at a depth of no more than 5 cm. In the soil, the larvae construct a chamber in the ground where they live until emergence (Delgado & Couturier 2000, 2004). The entire life cycle from

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egg until adult emergence lasts an average of 93.9 days (Delgado & Couturier 2004, O'Brien & Couturier 1995).

Several authors have claimed that seasonal flooding can kill these beetles (Perez & Innacone 2008, Penn 2006), but to date, there have been no experimental tests of whether these pests can survive flooding events. Here, we report the results of an experiment designed to test whether (1) flooding survival is associated with water circulation at different larval stages and (2) whether flooding tolerance has been lost in insect populations associated with host plant populations in non-flooded habitats.

Material and Methods

The study was conducted from December 2004 to May 2009. The first experiment was performed under natural conditions in a cultivated plantation of camu-camu (*M. dubia*) that was 7 years old, located in the community of Sapuena (03°73′S, 73°36′W), Municipality of Requena, Department of Loreto in the Peruvian Amazon.

The plantation is flooded annually by the Ucayali River for a period of 90–120 days (February to May) with inundation occurring up to 5.70 m in depth. In February, the water begins to cover the soil and reaches its peak in the month of April. During this period one finds extremely murky waters filled with Andean sediments, as well as frequent rainfall in the study site, suggesting that there is plenty of water circulation and high oxygen levels. At the end of the month of April, there is a period of approximately 15 days in which the water level stops rising until it begins to fall again in May, when soils are again above the water level. During this period, rainfall in the region is usually less frequent and there is more sunshine. As a result, temperature, CO_2, and primary productivity increase in the water, while the levels of oxygen decrease (Junk *et al* 1983).

At the beginning of the flooding season, we installed three cylindrical containers of 25 cm in height and 10 cm in diameter, with the bottom opening covered with a 1-mm^2 wire mesh. The vessel was attached by its base to the ground with the help of two sticks to avoid movement during the experiment. In each vessel, we placed 30 *M. dubia* fruits infested with larvae of *C. dubiae* representing various ontogenetic stages. This experiment was repeated three times.

The second experiment was conducted under laboratory conditions at the Research Institute of the Peruvian Amazon (IIAP) in the city of Iquitos. Two liters of plastic containers were prepared in which we deposited 1.2 L of water and 200 g of sand previously disinfected with sodium hypochlorite. Four trials were conducted under different conditions (described below): in each trial (which was repeated three times), we deposited 30 fruits with larvae of *C. dubiae* from the flooded cultivated field plots. In the first trial, larvae of different developmental stages were deposited in the containers where they remained under water for 77 days without any circulation of water. In the second, different larval stages were again deposited in the containers for 64 days under water, but the water was circulated every 48 h. Water was circulated by removing half the water from the container and replacing it with fresh water. However, larvae were submerged at all times in all the experiments. The third trial consisted of only the last instar larvae in the containers remaining under water for 78 days, with circulation of water every 48 h. The fourth trial was conducted under the same conditions as the third trial but was conducted with larvae collected from an experimental cultivated plot of *Myrciaria* that has been planted for over 25 years, located in terra firme soils. We analyzed all experiments together using a one-way analysis of variance (ANOVA) and assessed differences among trials using post hoc tests.

Results and Discussion

We found that the different experiments yielded statistically significant results using a one-way ANOVA ($F_{df=4, 10}=46.72$, $p=1.94E{-}06$). Survival in trial 1 was significantly lower than the other trials, and survival in trial 3 was significantly higher than the others (Fig 1).

In the experiment conducted under natural conditions, we collected the fruits 3 days after the water receded from the cultivated plots. The larvae remained under water for 93 days. On average, we found 21 fruits of the 30 placed in each container, and an average of 9 of these fruits (42.8%) contained living larvae. The rest of the larvae either died before reaching the last instar under water, or alternatively, others emerged from the fruit and buried themselves or were predated by fish.

Under laboratory conditions, larval survival in the flooded soil trial, ranged from 0% in containers that were not subjected to water circulation to up to 88.3% in containers including the last larval stage with circulation of water every 48 h (Fig 1). The experiments show that the earliest larval stages do not tolerate complete submersion very well, which would be one of the first factors that larvae must overcome to survive in these flooded zones. When comparing the levels of survival between larvae of different stages, backgrounds, and water circulation, we found that the survival of larvae from floodplains (the second trial) was higher than that of the terra firme (the fourth trial), but this was not statistically significant (Fig 1). Probably, the slight difference that we found is due to longer submersion and larger percentage of first instars of terra firme habitats that were included in the experiments rather than any evolutionary adaptation that differ between the two populations.
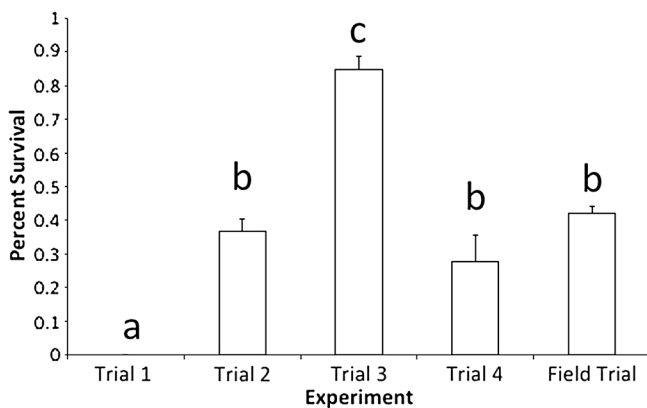
Fig 1 Percent survival of *Conotrachelus dubiae* for each of the five experiments, showing average survival and standard error. Trial 1 had no water circulation, trial 2 included water circulation and mixed larval life stages, trial 3 included water circulation but included only older larval life stages, trial 4 included water circulation and mixed larval life stages from the terra firme population, and the field trial shows the results from the experiment under field conditions. Significant differences among groups were tested by Tukey honestly significant difference (HSD) tests and are denoted by *different letters*

In the third trial, we included only the last instar larvae, and we observed significantly higher survival than all the other trials (Fig 1). In one of the replicates, 1 hour after the infested fruits were placed in containers, 41% of the larvae left the fruits, and after 6 hours, 82% had left. In both cases, all were found buried at a depth of about 2 cm. In a different replicate, only 35% of the larvae left the fruit, and of those which abandoned the fruit, 6.5% did not appear to bury themselves to undergo metamorphosis to the aquatic phase, exhibiting caudal movements during this period. Two hours after the water was withdrawn from the containers, these larvae began to move to begin their phase of transformation.

Seasonal flooding events in Amazonian ecosystems represent an important stress for terrestrial organisms living near rivers. *Conotrachelus dubiae* is one of the species associated with the plant *M. dubia*, which naturally occurs in seasonally flooded forests adjacent to blackwater rivers. At present, due to the cultivation of this plant in many environments, the beetle is found in soils flooded by both white and blackwater rivers and even in *terra firme* non-flooded soils (Delgado & Couturier 2004). *Conotrachelus dubiae* in its last stage of development can survive flooding for a period longer than 3 months at depths of 5 ft. This adaptation for surviving floods occurs in populations where it floods every year as well as in populations where there have been no flooding events for over 20 years.

Bachmann *et al* (1998), when studying both the phylogenetic relatedness and flooding adaptations of populations of the millipede *Pycnotropis epiclysmus* (Polydesmida: Platyrhacidae) from seasonally flooded soils (white water and mixed) and non-flooded soils, found that flood tolerance

does not partition the respective populations into genetically different ecotypes, concluding that flooded and non-flooded populations are ecologically adapted populations of the same species. When populations of *Brachyiulus bagnalli* (Diplopoda: Lulidae) that do not naturally inhabit flooded areas have been subjected to experimental flooding, it has been found that they can withstand flooding for several days (Zulka 1996), or for *Rostrozetes foveolatus* Sellnick (Sarcoptiformes: Haplozetidae), even for some months (Franklin *et al* 2001). If we consider the phenological period of *M. dubia* (Inga *et al* 2001, Rodrigues *et al* 2001, Maués & Couturier 2002) and the period of development of the immature state of *C. dubiae* (Delgado & Couturier 2004, Perez & Innacone 2008), it can be assumed that the species is univoltine in flooded soils and is multivoltine in terra firme habitats. When we monitored plantations of *M. dubia* of different ages in terra firme areas, we found fruits infested with larvae at different times of the year, confirming that the species may have more than two life cycles in terra firme habitats. Different life history strategies for the same species (univoltine or multivoltine) may be the result of a genetic adaptation to different environments for each population or alternatively represent a phenotypic plasticity response of populations to different environmental conditions (West-Eberhard 1989). The results of our experiments suggest that the responses of the larvae of *C. dubiae* to seasonal flooding are due to ecological plasticity of the species, rather than due to different ecological adaptations from genetically distinct populations. Howewer, it is important to note that the terra firme insects that we used in our experiment were from a plantation that is only 25 years old. It is possible that not enough generations have elapsed for the populations to become locally adapted to non flooded conditions.

Several authors state that the lack of oxygen in water is the main factor that organisms must overcome to survive in flooded environments, regardless of the duration or the geographic region of the flooding event (Adis & Junk 2002, Plum 2005). In the flooded forests of the Amazon, the scarcity of oxygen in the water can reach conditions of anoxia (Junk *et al* 1983), and in the lower Rio Ucayali, this occurs in the months of April and May, with the apex of the increase of river levels (precipitation decreases, water stays at its same level), which is accompanied by higher insolation, temperature increases, and high metabolic activity caused by decomposition of organic matter by microorganisms. Oxygen deficiency can be overcome in several ways. Some species, such as *B. bagnalli* (which does not inhabit flooded areas), when subjected to experimental flooding, are able to obtain oxygen by diffusion through their cuticle, while those that normally live in flooded habitats such as *Polydesmus denticulatus* (Polydesmida: Polydesmidae) can uptake oxygen using spiracle plastron structures (Zulka 1996). *Gonographis adisi* Hoffmann (Polydesmida: Pyrgodesmidae)

lives submerged in water for 11 months of the year thanks to a hydrophobic layer on the cuticle, which enables plastron respiration (Hoffman 1985, Adis 1986). Hoback *et al* (1998), studying the larvae of the tiger beetle, *Cicindela togata* LaFerté-Sénectère (Coleoptera: Cicindelidae), found that this species can live up to 6 days under conditions of complete anoxia. Terrestrial insects such as *C. togata*, facing increasing hypoxia, become quiescent, decrease their aerobic metabolic rate, and switch to anaerobic metabolism (Hochachka 1980, Hoback & Stanley 2001). Maitland & Maitland (1994) have documented that tunnels built by invertebrates in the water are used to capture air, but these tunnels require 1 to 2 days to complete (until the gallery is saturated with water), so these organisms also need tracheal gills to facilitate oxygen uptake.

We dissected several larvae of *C. dubiae*, and we did not find any plastrons or any similar structures. Thus, we assume that the larva of *C. dubiae* obtains oxygen from the water by diffusion assisted by caudal movements. It is less likely that these insects have shifted to an anaerobic metabolic pathway, which would mean total paralysis of the individual. Instead, the larva of *C. dubiae* exhibits several different types of movements and other behaviors during and immediately after the flood simulation. The species in their larval stage do not need to invest in major physiological changes to survive seasonal flooding, instead restricting certain metabolic activities, as has been suggested for other groups of invertebrates (Adis & Messner 1991, Adis 1992, Zerm *et al* 2001, Adis & Junk 2002). The building of underground burrows to store air and facilitate oxygen uptake is discarded as a possibility, because the larvae remain under water for long periods and because we observed that many larvae did not need to be buried to survive flooding.

Conotrachelus dubiae is currently distributed throughout the Amazon basin where its host plant occurs in soils flooded by blackwater and whitewater rivers and in non-flooded soils, in naturally occurring and cultivated plantations. The larvae survive seasonal floods submerged in water for a period of 3–5 months. Populations in non-flooded areas exhibited the same degree of flood tolerance as populations from flooded habitats, suggesting that flood tolerance is an ecologically plastic trait. The most important factor driving larval survival in flooding is the developmental stage at the time that the larvae are covered by water. The larvae of the last stage have a greater chance of survival. We suspect that the exchange of oxygen takes place in the integument, aided by caudal movements. Individuals enter a state of dormancy in their last developmental stage, ceasing certain metabolic activities related to food.

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