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Survival and fecundity of Dichroplus maculipennis and Ronderosia bergi (Orthoptera: Acrididae: Melanoplinae) following infection by Beauveria bassiana (Ascomycota: Hypocreales) under laboratory conditions.

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Survival and fecundity of *Dichroplus maculipennis* and *Ronderosia bergi* (Orthoptera: Acrididae: Melanoplinae) following infection by *Beauveria bassiana* (Ascomycota: Hypocreales) under laboratory conditions.

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Abstract

This study examined the effects of strain *Beauveria bassiana* (LPSC 1067) on nymphal development time, fecundity, and adult survival in *Dichroplus maculipennis* and *Ronderosia bergi* under laboratory conditions. It was observed that infection with $1x10^3$ conidia/ml altered nymphal development time, fecundity, and adult survival in both species. Mortality of *D. maculipennis* during third through the last instar (sixth) was significantly higher among treated nymphs (66 $\pm$ 3.8 %) than in controls (15 $\pm$ 1.7 %). Similarly, mortality in *R. bergi* during third through the last instar (fifth) was higher in treated nymphs (71 $\pm$ 2.8 %) than in controls (19 $\pm$ 1.5 %). Nymphal development times of both infected *D. maculipennis* and *R. bergi* were longer than controls. On the other hand, among survivors of both species, control adults lived longer than infected adults. Finally, control grasshoppers of both species were much more successful reproductively than infected grasshoppers.

Keywords: *Dichroplus maculipennis*; *Ronderosia bergi*; Orthoptera: Acrididae; Grasshoppers; *Beauveria bassiana*; Entomopathogenic fungi

Introduction

As in other temperate grasslands of the world, grasshoppers are among the most important native herbivores throughout the Argentine Pampas and parts of Patagonia (Mariottini et al. 2011a). The economic importance as agricultural pests of these insects has been recognized in the country since the mid-to-late XIX century, and outbreaks of different species are a recurring phenomenon (Lange et al. 2005). The melanopline grasshoppers selected for this study are two of the 18 grasshopper species considered as economically important in Argentina (Cigliano et al. in press). *Dichroplus maculipennis* (Blanchard) is a characteristic univoltine species of grasshopper communities in areas of the Pampas and Patagonia regions (Lange and Azzaro 2008; Mariottini et al. 2011a). *Ronderosia bergi* (Stål) is a common species with no obligatory embryonic diapause (i.e., multivoltine capacity) in natural grasslands and crops in areas of central and northern Argentina (Mariottini et al. 2010). Both species have a wide geographic distribution, occurring in southernmost Brazil, much of Argentina and Chile, and Uruguay (Mariottini et al. 2006, 2011a). Although an introduced biocontrol agent, the microsporidium *Paranosema locustae* (Canning), is known to be established in parts of the Pampas and Patagonia (Lange and Azzaro 2008; Bardi et al. 2012), chemical insecticides are still the only available option for grasshopper control in the country, but their use is of significant environmental concern (Goldstein et al. 1999; Jergentz et al. 2005; González et al. 2010).

Fungal entomopathogens are important biological control agents worldwide and have been the subject of intense research for more than 100 years (Vega et al. 2012). While the majority of studies assessing pathogens as biocontrol agents deal with their ability to produce mortality in the target pest (Blanford and Thomas 2001), previous studies have indicated that infection with the fungal entomopathogen *Metarhizium*

acridium (Driver & Milner) can reduce feeding and flight ability in the desert locust *Schistocerca gregaria* Forskal (Moore et al. 1992; Seyoum et al. 1994). However, sublethal effects produced by entomopathogenic fungi on grasshoppers that survive to infection have been seldom addressed.

As in other insects, some features of the life cycle of grasshoppers like longevity, reproduction, and development time, among others, are key factors in the population dynamics of the different species (Joern and Gaines 1990). Understanding the influence of the pathogen on the developmental period and reproduction of an insect pest is important in order to develop a successful biocontrol agent. Therefore, the aim of this study was to evaluate the influence of *Beauveria bassiana* (LPSC 1067) (Ascomycota: Hypocreales) on nymphal development time, fecundity, and adult survival in the pest grasshoppers *D. maculipennis* and *R. bergi* under laboratory conditions.

Material and Methods

Collection of insects

Adult males and females of *D. maculipennis* and *R. bergi* were collected with insect l nets in natural and improved pastures at the locality of Laprida (36º 02`S, 59º 06`W), Buenos Aires province, in the southern Pampas region as defined by Morrone (2006). Once in the laboratory, grasshoppers were kept following general procedures as described by Henry (1985). Individuals of both sexes were placed in wire-screened, aluminium cages (20x20x30 cm) in a rearing room under controlled conditions (30ºC, 14L:10D, 40% RH) routinely used worldwide (Hinks and Erlandson 1994; Mariottini et al. 2010, 2011). Grasshoppers were fed daily with thoroughly washed, fresh leaves of a variety of grasses, lettuce, cabbage, and wheat bran flakes. Each cage was provided with substrates for egg-pod laying that consisted of plastic containers (10 cm deep) filled

with sterilized sand. Thermoregulation and mating was stimulated with 75W bulbs suspended 15 cm above each cage. Grasshoppers were maintained until death when they were immediately examined by dissection (Lange, 1996) or incubated in humid chambers (Lacey and Brooks 1997) to check for their sanitary condition. Nymphs emerging from the resulting egg-pods were used in the laboratory assays. This procedure was carried out separately for both species of grasshoppers.

Fungal isolate

The fungal strain used was *B. bassiana* (LPSC 1067) from the culture collection of the Spegazzini Institute (LPSC), La Plata, Argentina. The choice of this fungal strain was based on its laboratory efficacy against other pest grasshopper and locust species of Argentina (Pelizza et al. 2012 a, b). Conidia of the fungal strain were obtained from cultures on potato-dextrose-agar medium after incubation for 10 days at 25 ºC in darkness.

Preparation of conidial suspension

Conidia were harvested with disposable cell scrapers (Fisherbrand®) from 10-day-old cultures and placed in test tubes containing 0.01% (v/v) Tween 80® (polyoxyethylene sorbitan monolaurate) (Merck). Suspensions were vortexed for 2 min, filtered through four layers of sterile muslin, and adjusted to $1x10^3$ conidia/ml according to Blanford and Thomas (2001) after cell counting in a Neubauer haemocytometer. Viability of the fungal conidia was determined after 24 h as described by Lane et al. (1988). This germination test was repeated for each stock suspension to maintain the fidelity of the viability assessments.

Insect rearing and inoculation procedure

At hatching, groups of five nymphs were placed in acetate tubes (50 cm long x 9 cm diameter) (Henry 1985). After the first moult, grasshoppers were kept individually in similar cages, but smaller (20 cm long x 10 cm diameter). After the second moult, 161 and 200 individuals of *D. maculipennis* and *R. bergi*, respectively were inoculated individually by spraying with 1 ml of a conidial suspension of $1x10^3$ conidia/ml(in 0.01% [v/v] Tween 80®). As control groups, 60 and 66 individuals of *D. maculipennis* and *R. bergi*, respectively were used. The controls were sprayed in the same fashion, but with 1 ml of 0.01% [v/v] Tween 80® only. After inoculation, grasshoppers were kept under controlled conditions (30ºC, 14L:10D, 40% RH) while nymphal development time, fecundity, and adult survival were monitored and recorded on a daily basis. Immediately after moulting to adults, females and males of both species were separated into couples (1♂, 1♀). Each couple was placed in a wire-screened, aluminium cage (12x12x16cm). Survival and egg production (number of eggs per pod) by pairs were monitored and recorded throughout their lives. Dead grasshoppers were removed and immediately deposited in high-humidity chambers (sterile Petri dishes with filter paper dampened with sterile distilled water). Mycosis was confirmed by microscopically examination of the dead grasshoppers.

Statistical analyses

An Analysis of Variance (model I) with accordance Bifactorial was performed to determine if differences were significant between controls and treatments at each nymphal stage evaluated for both *R. bergi* and *D. maculipennis*. This model evaluates the species*treatment interaction. The variable used was numbers of days lived by species evaluated to each nymphal stage. The parameters for Anova test were analyzed. For later comparisons a Tukey test for unbalanced data was used.

A unifactorial Anova test was used for evaluation of the significant difference between treatment and control of sixth stage of *D. maculipennis.* A Model I multifactorial Anova test (species, treatment, sex) was used for evaluation of the number of days lived for both species since adulthood to death. Data were transformed by square root and then analyzed by Anova and Tukey tests (interaction sex*treatment for each grasshoppers species). Analysis of Variance with accordance bifactorial and multifactorial Anova test were performed with the software Version InfoStat 2007 (InfoStat 2001).

Results

Inoculation of grasshoppers with the fungus significantly altered nymphal development time, fecundity, and adult survival of both grasshopper species,. Mortality in *D. maculipennis* during third through sixth instars was substantially higher among treated nymphs (66 $\pm$ 3.8 %) than in controls (15 $\pm$ 1.7 %). Similarly, mortality in *R. bergi* during third through fifth instar was higher in treated nymphs (71 $\pm$ 2.8 %) than in controls (19 $\pm$ 1.5 %). More than 95 % of dead grasshoppers exhibited outward growth of the fungus after 24 h under humidity chamber incubation. Likewise, nymphal development times of infected *D. maculipennis* and *R. bergi* were longer than in controls, (Table 1).

Statistical analysis showed that for third and fourth instars, differences were highly significant ($p<0.01$) for species and treatment factors, but the interaction between these was not significant ($p>0.001$) (Table 2). Not significant differences ($p>0.01$) were observed for the Species factor in fifth stage. The treatment factor and the interaction between both factors (Species*treatment) were highly significant ($p<0.01$) (Table 2). The sixth instar of *D. maculipennis* showed highly significant difference ($p<0.01$) between treated and controls.

Among survivors of both species, control adults lived considerably longer that treated adults (Figures 1 and 2). Statistical analysis showed that for the evaluation of the number of days lived for both species from adulthood to death, only the interaction treatments*sex was not significant ($p>0.01$), being significant all others interactions ($p<0,01$) (Table3).

In the interaction sex*treatment of *D. maculipennis*, a highly significant difference ($p<0.01$) for treatment and sex factor was observed, but not for their interaction (treatment*sex) ($p>0.01$) (Table 4, Fig 1).

In the interaction sex*treatment of *R. bergi*, significant differences for the two factors and their interactions were observed ($p<0.01$), (Table 4, Figure 2).

Finally, control grasshoppers of both species were much more successful reproductively than infected grasshoppers. While infected females of both species did not copulate or lay eggs, control females of *D. maculipennis* and *R. bergi* copulated and laid on average 22.35 $\pm$ 3.82 and 13.08 $\pm$ 2.08 eggs per pod, respectively.

Discussion

This is the first report on sub-lethal effects caused by the entomopathogenic fungus *B. bassiana* on *D. maculipennis* and *R. bergi* of Argentina. Our results showed that insects of both species that were inoculated with *B. bassiana* (LPSC 1067) had a higher mortality in each of the different nymphal stages when compared with control grasshoppers. Similar results were observed by Blanford and Thomas (2001) on the Desert locust *Schistocerca gregaria* Forskal after inoculation with a sublethal dose of *M. anisopliae var. acridum.* Furthermore, we observed that nymphal development was prolonged in treated grasshoppers of both species as compared with controls. Although the entomopathogenic fungus *B. bassiana* prolonged the number of days that grasshoppers lived as nymphs, it is important to note that insects fed very little and were

lethargic as observed by Sanehdeep et al. (2011) in larvae of *Spodoptera litura* (Fabricius) infected with *B. bassiana.* Hernández et al. (2007) also observed a reduction of feeding of *Schistocerca piceifrons* (Walker) following infection by *Metarhizium anisopliae* var. *acridum*. Freimoiser et al. (2003) mentioned that the reduction of feeding, movement, and flying abilities of grasshoppers is related to the development and colonization of the fungus rather than toxin production.

In relation to fecundity, females of *D. maculipennis* and *R. bergi* infected with *B. bassiana* did not copulate or lay eggs while control females of both species did with no apparent drawbacks as reported by Mariottini et al. (2010, 2011b). Similar results were observed by Hornbostel et al. (2004) after applying a sublethal dose of *M. anisopliae* on engorged larvae of *Ixodes scapularis* Say.

Finally, grasshoppers of both species infected with *B. bassiana* in our study were shorter lived as adults compared with the controls. Gindin et al. (2006) reported decreased longevity of red palm weevil females due to fungal infection with *B. bassiana* and *M. anisopliae*. However, our results differ from those obtained by Blanford and Thomas (2001) who found no difference in survival and fecundity of adults of *S. gregaria* infected with the fungus *M. anisopliae* with respect to controls. This difference with our study might be related to the fact that they applied a sublethal dose of the fungus *M. anisopliae* on adults and newly fledged locusts. One possible hypothesis that would allow understanding why the grasshoppers infected with *B. bassiana* have lower survival and fecundity than controls is suggested by Sanehdeep et al. (2011). They proposed that the fungal infected insects may have acquired and stored less nutrient resources than that of control insects which might have affected the longevity and fecundity of females. This is supported by Khachatourians (1986) who suggests that entomopathogenic fungi caused the death of their host due to exhaustion of nutrients

and liberation of toxins in the hemolymph. So, nutritional deficiency and toxins acting separately or in unison can drastically affect the development of an insect, especially reproduction and molting which have high energetic demands.

From a control point of view, sublethal effects caused by *B. bassiana* on *D. maculipennis* and *R. bergi* populations may be important if, for instance, strain *B. bassiana* (LPSC 1067) is combined with "biorational" insecticides for some rapid knockdown while retaining the advantages of the residual effects of the fungus.

Even though further studies are needed, particularly under natural conditions, our results suggest that strain *B. bassiana* (LPSC 1067) has potential as a control agent for *R. bergi* and *D. maculipennis* by substantially increasing mortality during third through fifth and sixth instars, respectively, and preventing mating and oviposition by both species.

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References

Blanford, S., and Thomas, M.B. (2001), 'Adult survival, maturation and reproduction of the desert locust *Schistocerca gregaria* infected with the fungus *Metarhizium anisopliae* var *acridum'*, *Journal of Invertebrate Pathology*, 78, 1-8.

Bardi, C., Mariottini, Y., Plischuk, S. and Lange, C.E. (2012), 'Status of the alien pathogen *Paranosema locustae* (Microsporidia) in grasshoppers (Orthoptera: Acridoidea) of the Argentine Pampas' *Biocontrol Science and Technology*, 22, 497-512.

Cigliano, M.M., Pocco, M.E., and Lange C.E. (in press), 'Acridoideos (Orthoptera) de importancia agroeconómica. in *Biodiversidad de Artrópodos Argentinos*, eds. Roig-Juñent S, L. E. Claps and J. J. Morrone, Sociedad Entomológica Argentina. pp, 1-26.

Freimoser, F.M., Screen, S., Savita, B., Hu, G. and St Leger, R.J. (2003), 'Expressed sequence tag (EST) analysis of two subspecies of *Metarhizium anisopliae* reveals a plethora of secreted proteins with potential activity in insect hosts', *Microbiology*, 149, 239-247.

Gindin, G., Levski, S., Glazer, I., and Soroker, V. (2006), 'Evaluation of the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* against the red palm weevil *Rhynchophorus ferrugineus*', *Phytoparasitica*, 34, 370-379.

Goldstein, M.I., Lacher, T.E., Woodbridge, B., Bechard, M., Canavelli, S.B., Zacagnini, M.E., Cobb, G., Scollon, E.J., Tribolet, R., and Hooper, M. (1999), 'Monocrotophos-induced mass mortality of Swainson´s hawks in Argentina', *Ecotoxicology*, 8, 201-214.

Gonzalez, M., Miglioranza, K.S., Aizpún, J.E., Isla, F.L., and Peña, A. (2010), 'Assessing pesticide leaching and desorption in soils with different agricultural activities from Argentina (Pampa and Patagonia)', *Chemosphere*, 81, 351-358.

Henry, J.E. (1985), 'Melanoplus spp.', in '*Handbook of Insect Rearing vol. 1*', eds. P. Singh and R.F. Moore, Amsterdam: Elsevier, pp 451-464

Hinks, C.F. and Erlandson, M.A. (1994), 'Rearing grasshoppers and locusts: Review, rationale and update', *Journal of Orthoptera Research*, 3, 1-10.

Hornbostel, V.L., Ostfeld, S.R., Zhioua, E., Benjamin, M.A. (2004), 'Sublethal effects of *Metarhizium anisopliae* (Deuteromycetes) on engorged larval, nymphal, and adult *Ixodes scapularis* (Acari: Ixodidae)', *Journal of Medical Entomology*, 41(5), 922-929.

InfoStat. 2001. Manual del usuario, versión 1. Universidad Nacional de Córdoba. Córdoba, Argentina.

Jergentz, S., Mugni, H., Bonetto, C., and Schulz, R. (2005), 'Assessment of insecticide contamination in runoff and stream water of small agricultural streams in the main soybean area of Argentina', *Chemosphere*, 61, 817–826.

Joern, A., and Gaines, S.B. (1990), 'Population dynamics and regulation in grasshoppers', in *Biology of grasshoppers*, eds. John Wiley & Sons, New York, pp 415-482.

Khachatourians, G.G. (1986), 'Production and use of biological pests control agents' *Trends in Biotechnology*, 4, 120-124.

Lacey, L.A., and Brooks, W.M. (1997), 'Initial handling and diagnosis of diseased insects', in *Manual of techniques in Insect Pathology*, eds. Lacey, L.A., and Brooks, W.M. Academic, San Diego, pp1-15.

Lane, B.S., Humphreys, B.S.A.M., Thompson, K., and Trinci, A.P.J. (1988), 'ATP content of stored spores of *Paecilomyces farinosus* and the use of ATP as criterion of spore viability', *Transactions of the British Mycological Society*, 90, 109-111.

Lange, C.E. (1996), 'Protistas patógenos de insectos terrestres', in *Microorganismos patógenos empleados en el control microbiano de insectos plaga*, ed. R. Lecuona Buenos Aires (Argentina), Mariano Mas, pp. 87-104.

Lange, C.E., Cigliano, M.M. and De Wysiecki, M.L. (2005), 'Los acridoideos (Orthoptera: Acridoidea) de importancia económica en la Argentina', in *Manejo integrado de la langosta centroamericana (Schistocerca piceifrons piceifrons, Walker) y acridoideos plaga en América Latina,* eds. L. Barrientos-Lozano and P. Almaguer Sierra, Instituto Tecnológico de Ciudad Victoria, Tamaulipas, México, pp 93-135.

Lange, C.E. and Azzaro, F.G. (2008), 'New case of long-term persistence of *Paranosema locustae* (Microsporidia) in melanopline grasshoppers (Orthoptera: Acrididae) of Argentina' *Journal of Invertebrate Pathology,* 99, 357-359

Mariottini, Y., De Wysiecki, M.L. and Lange, C.E. (2006), 'Desarrollo postembrionario de *Ronderosia bergi* (Orthoptera: Acrididae: Melanoplinae) bajo condiciones controladas', *Revista Sociedad Entomologica Argentina*, 65 (1-2), 81-85.

Mariottini, Y., De Wysiecki, M.L. and Lange, C.E. (2010), 'The biology and population parameters of the grasshoppers *Ronderosia bergi* (Stal) (Orthoptera: Acrididae: Melanoplinae), under laboratory conditions', *Journal of Insect Science*, 10: 109.

Mariottini, Y., De Wysiecki, M. L., and Lange, C. E. (2011a), 'Seasonal occurrence of life stages of grasshoppers (Orthoptera: Acridoidea) in the southern pampas, Argentina', *Zoological Studies*, 50 (6), 737-744.

Mariottini, Y., De Wysiecki, M.L. and Lange, C.E. (2011b), 'Longevity and fecundity of *Dichroplus maculipennis* (Orthoptera: Acrididae) at non-outbreaking and outbreaking situations', *Revista Brasileira de Entomología*, 55(3), 435-438.

Moore, D., Reed, M., Le Patourel, G., Abraham, Y.J. and Prior, C. (1992), 'Reduction of feeding by the desert locust, *Schistocerca gregaria*, after infection with *Metarhizium flavoviride*', *Journal of Invertebrate Pathology*, 60, 304-307.

Morrone, J.J. (2006), 'Biogeographic areas and transition zones of Latin America and the Caribbean islands based on panbiogeographic and cladistic analyses of the entomofauna', *Annual Review of Entomology*, 51, 467-494.

Pelizza, S.A., Eliades, L.A., Scorsetti, A.C., Cabello, M.N. and Lange, C.E. (2012a), 'Entomopathogenic fungi from Argentina for the control of *Schistocerca cancellata* (Orthoptera: Acrididae) nymphs: fungal pathogenicity and enzyme activity', *Biocontrol Science and Technology*, 22(10), 1119-1129.

Pelizza, S.A., Eliades, L.A., Saparrat, M.C.N., Cabello, M.N., Scorsetti, A.C. and Lange, C.E. (2012b), 'Screening of Argentine native fungal strain for biocontrol of the grasshoppers *Tropidacris collaris*: relationship between fungal pathogenicity and chitinolytic enzyme activity', *World Journal of Microbiology and Biotechnology*, 28, 1359-1366.

Sanehdeep, K., Harminder, P.K., Kirandeep, K., Amarjeet, K. (2011), 'Effect of different concentrations of *Beauveria bassiana* on development and reproductive potential of *Spodoptera litura* (Fabricius)', *Journal of Biopesticides*, 4(2), 161-168.

Seyoum, E., Moore, D. and Charnley, A.K. (1994), 'Reduction in flight activity and food consumption by the desert locust, *Schistocerca gregaria*, Forskal (Orth., Cyrtacanthacrinae), after infection with *Metarhizium flavoviride*' *Journal Applied Entomology*, 118, 310-315.

Story, P. and Cox, M. (2001), 'Review of the effects of organophosphorus and carbamate insecticide on vertebrates. Are there implications for locust management in Australia', *Wildlife Research*, 28, 179-193.

Tibebu, H., Landin, J., Wennergen, U. and Bergman K.O. (1995), 'Life table for the grasshoppers, *Aiolopus longicornis*, under laboratory conditions and demographic effects of the pathogen *Nosema locustae*', *Biological control*, 5, 497-502.

Vega, F.E., Meyling, N.V., Luangsa-ard, J.J., and Blackwell, M. (2012), 'Fungal entomopathogenes', in Insect Pathology, eds. F.E. Vega and H.K. Kaya, London: Elsevier, pp. 171-220

Hernández, V.M.., Padilla, A.B. and Toriello, C. (2007), 'Reduction of feeding by *Schistocerca piceifrons piceifrons* (Orthoptera: Acrididae), following infection by *Metarhizium anisopliae* var. *acridum*', *Florida Entomologist*, 90, 4

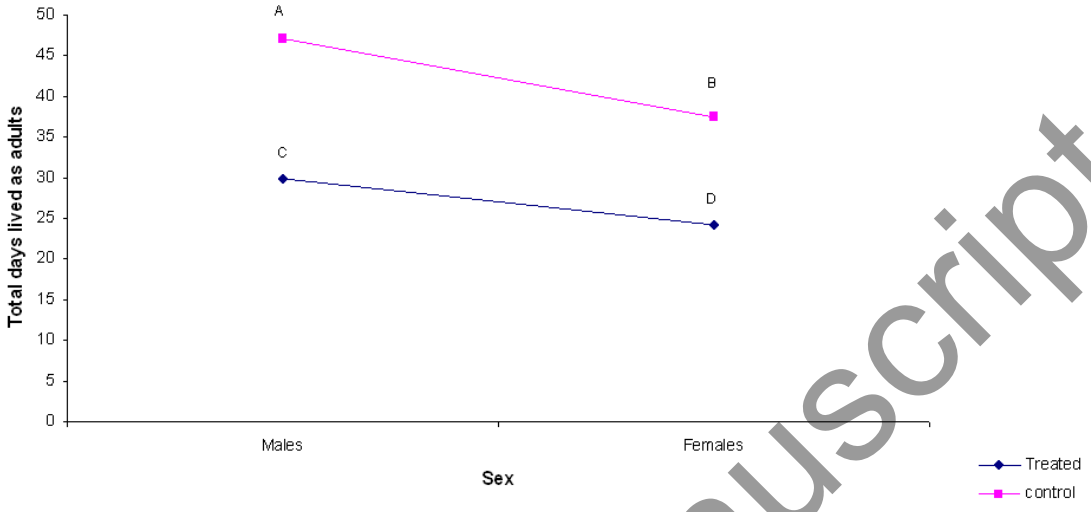

Fig. 1: Total days lived as adults for males and females of *Dichroplus maculipennis* treated and control. Different letters indicate significant differences according to the Tukey test (α=0.01).

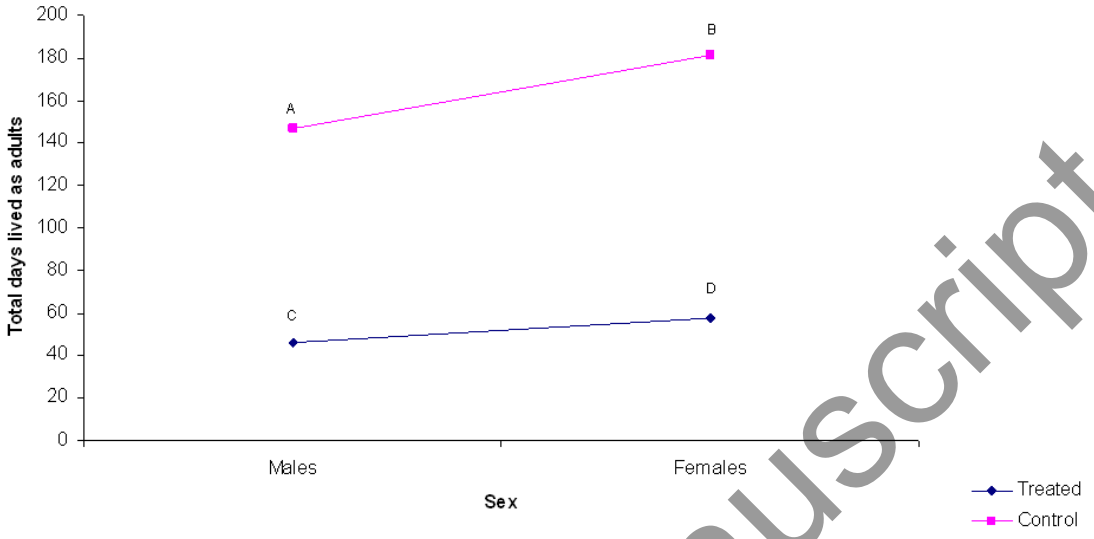

Fig. 2: Total days lived as adults for males and females of *Ronderosia bergi* treated and control. Different letters indicate significant differences according to the Tukey test (α=0.01).

Table 1: Number of days lived by *Dichroplus maculipennis* and *Ronderosia bergi* (mean ± SD) in each nymphal instar, at 30ºC and 14:10 L.D. Number of days lived followed by the same letter are not significantly different according to the Tukey test for unbalanced data ($\alpha = 0.01$). CV= Coefficient of variability.

Species	**Treatment**	**Third instar**	**Fourth instar**	**Fifth instar**	**Sixth instar**
		CV=16.11 N=324	CV=15.59 N=250	CV=15.86 N=231	CV=16.84 N=106
R. bergi	Treated	13 ± 1.3 a	14 ± 1 a	18 ± 1.9 a	-
R. bergi	Control	7 ± 0.8 c	8 ± 1.2 c	9 ± 1.1 d	-
D. maculipennis	Treated	12 ± 2.4 b	15 ± 2 b	17 ± 3.4 b	19 ± 2.8 a
D. maculipennis	Control	7 ± 1.8 c	8 ± 2.4 c	10 ± 1.4 c	13 ± 2.5 b

Table 2: Results of Analysis of Variance (ANOVA) for species factor, treatment factor and the interaction between both factors (Species*treatment).

	Third instar			**Fourth instar**			**Fifth instar**			**Sixth instar**		
	DF	F value	P	DF	F value	P	DF	F value	P	DF	F value	P
Species	1	13.01	<0.0004	1	17.61	<0.0001	1	0.03	0.8580	1	124.55	<0.0001
Treatment	1	881.35	<0.0001	1	744.32	<0.0001	1	802.87	<0.0001			
Specie* Treatment	1	2.37	0.1248	1	0.70	0.4034	1	16.78	<0.0001			

Table 3: Results of Analysis of Variance (ANOVA) for the evaluation the numbers of days lived for *Dichroplus maculipennis* and *Ronderosia bergi* since adulthood to death.

	DF	F value	P value
Species	1	4563.78	<0.0001
Treatment	1	3151.11	<0.0001
Sex	1	10.71	0.0012
Species*Treatment	1	1222.58	<0.0001
Species*Sex	1	198.76	<0.0001
Treatment*Sex	1	1.77	0.1852
Species*treatment*Sex	1	10.22	0.0016

Table 4: Results of Analysis of Variance (ANOVA) for the interaction sex * treatment of *Dichroplus maculipennis* and *Ronderosia bergi*.

	D. maculipennis			***R. bergi***		
	DF	**F value**	**P value**	**DF**	**F value**	**P value**
Treatment	1	110.38	<0.0001	1	35984.85	<0.0001
Sex	1	28.87	<0.0001	1	1308.30	<0.0001
Treatment*Sex	1	0.86	0.3560	1	88.85	<0.0001