The Toxicity of Used Coffee Grounds to the Larvae of *Ochlerotatus (Finlaya) notoscriptus* (Skuse) (Diptera: Culicidae)

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The exotic Ochlerotatus notoscriptus *is the most widespread container-breeding mosquito species in anthropic habitats in northern in New Zealand, and also a potential disease vector. We tested the toxicity of used coffee grounds, a recently proposed larvicide, to the species' larvae, which is known to be tolerant to a wide range of water quality levels. High concentrations of used coffee grounds induced very large larval mortality within a few days. However, large mortality occurred in the controls after two weeks, and there was an apparent boost to larval survivorship in treatments with low to medium concentrations of coffee grounds. Although the results suggest that used coffee grounds could constitute a cost-free and environmentally sound alternative to less desirable insecticides, we believe that extensive field trials are necessary before their use as a larval control method is advocated.*

Key words: used coffee grounds - *Ochlerotatus notoscriptus* - larvae – toxicity

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The biological effects of caffeine have been known for some time. Caffeine was shown to have deleterious effects on mammals, inducing changes of sex ratio in Chinese hamsters *Cricetulus griseus* (Weathersbee et al. 1975) and interfering with foetus development of Wistar rats *Rattus norvegicus* (Smith et al. 1987). It induced high mortality in the fruit fly *Drosophila melanogaster* (Diptera: Drosophilidae) (Nigsch et al. 1977; Zimmering et al. 1977) and the moth fly *Telmatoscopus albipunctatus* (Sehgal et al. 1977), and it also slowed down growth and development in the latter species. Caffeine inhibits oviposition and delayed development

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of the shot-hole borer beetle *Xyleborus fornicatus* (Hewavitharanage et al. 1999). Studies on *Drosophila prosaltans* demonstrated a series of deleterious biological effects (Itoyama & Bicudo 1992, 1997), which lead to the suggestion of caffeine's potential usefulness as an alternative insect control method.

A study in Brazil has indicated that caffeine negatively affects the development of *Aedes (Stegoymyia) aegypti* L. (Laranja et al. 2003). Though no field trials have yet been carried out, public health authorities in Brazil have begun advocating the application of used coffee grounds (UCG) as a larval control method against *Ae. aegypti*. If UCG are indeed an efficient larval control method, it could be also useful in the New Zealand scenario.

Ochlerotatus (Finlaya) notoscriptus (Skuse) is of public health significance in New Zealand (Derraik 2005; Derraik & Calisher 2004). This species is a vector of canine heartworm (Russell & Geary 1996), most likely a vector of Ross River virus in urban areas (Doggett & Russell 1997; Russell 1995; Watson & Kay 1997), and it is also a potential vector of Barmah Forest virus (Doggett & Russell 1997; Watson & Kay 1999). Therefore, UCG could be a valuable tool towards its control in anthropic environments.

Moreover, the relevance of testing the toxicity of UCG to *Oc. notoscriptus* larvae is the species' tolerance to a wide range of water quality levels. It has been found to be capable of breeding in clean water and in putrid and polluted solutions (e.g. Derraik 2004a,b). Thus, it is important to assess whether the deleterious effect of UCG on mosquito larval development is restricted to 'clean water species', such as *Ae. aegypti*.

This study therefore aimed to assess the toxicity of UCG to the larvae of *Oc. notoscriptus*. In addition to addressing larval survivorship, potential changes in the sex ratio of successfully hatched *Oc. notoscriptus* adults were also investigated. Note that since UCG may have organic compounds that may benefit mosquito larva, just testing the toxicity of caffeine per se does not allow for any conclusions on the actual usefulness of UCG as a potential larval control method.

MATERIALS AND METHODS

A large number of *Oc. notoscriptus* larvae were collected from a concrete drinking trough in the Wenderholm Regional Park, North Auckland (36° 32' 30" S, 174° 42' 35" E). The trough was located under the partial shade of some large trees, along a minor gravel track.

A medium strength Colombian coffee (from Robert Harris, New Zealand) was selected for the experiment, and preparation of UCG was carried out using standard household

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appliances. Water was boiled in an electric jug, and the coffee was prepared in a plunger. Five 15 ml standard tablespoons of coffee (c.25g of coffee) were placed into the plunger with one litre of boiling water. After exactly three minutes the water was extracted and the coffee grounds removed. Solutions at four different concentrations were prepared in 100 ml of water using the grounds (wet weight): 0.01, 0.05, 0.10 and 0.50 g/ml, which for the sake of clarity are referred to as UCG 0.01, UCG 0.05, UCG 0.10 and UCG 0.50, respectively. The equivalent caffeine concentrations in the grounds for each container at the start of the experiment were approximately 0.015, 0.075, 0.15 and 0.75 mg/ml, respectively. The controls had no grounds added to them. Voucher samples of UCG and solutions after 5 and 10 days of immersion were analysed by High Performance Liquid Chromatography (HPLC) for their respective caffeine content at the Cawthron Institute (Nelson, New Zealand).

Two types of medium were used for the solutions: the original (relatively clear) trough water from which the larvae were collected, and tap water with added dry sheep manure at 0.5 g/L. The latter solution was found to me an effective medium for rearing *Oc. notoscriptus* larvae in the field (Derraik & Slaney 2005), and the nutrient concentrations of nitrogen, phosphorus and potassium in the solution were approximately 0.015, 0.010 and 0.020 g/L, respectively.

The larvae were separated into two groups: $1^{st}/2^{nd}$ instars, and $3^{rd}/4^{th}$ instars. Forty larvae from the $1^{st}/2^{nd}$ instars group and 20 larvae from the $3^{rd}/4^{th}$ instars group were placed into the above solutions (control and four treatments for both water types). There were 6 replicates of each treatment, giving a total of 3,600 larvae. The transparent plastic cups containing the replicates were randomly arranged over a bench inside a laboratory with no air temperature control. All containers were covered with a plastic insect mesh in order to contain any hatched adults. Each replicate was checked daily for adults, while larval survivorship was checked every five days when numbers of larvae per container were counted. Data were analysed using binary logistic regressions, and the significance level used in all analyses was $P < 0.05$.

RESULTS

The survivorship curve for all combined data indicated a very large mortality of larvae in the first 5 days in all treatments, except the control (Fig. 1). By the 10^{th} day, the majority of larvae in the three highest caffeine concentrations had died (Fig. 1). Although there was no sudden larval mortality in the controls, there was a steady decline in survivorship over the first

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month. The mortality pattern for the UCG 0.01 treatment as shown by the survivorship curve (Fig. 1) was quite different from the others. At UCG 0.01 it took 43 days for mortality to reach 50%, while for the control UCG 0.05, UCG 0.10 and UCG 0.50 it took 15, 7, < 5 and < 5 days, respectively.

The initial results from the experiment (after 5 days) indicated that the higher the concentration of UCG, the higher the mortality rate of *Oc. notoscriptus* larvae, which was significantly lower in the control than in all treatments ($P < 0.001$; Fig. 1). By the 10th day, larval mortality in the control was still significantly lower ($P < 0.001$) than in all but UCG 0.01 ($P = 0.396$; Fig. 1). After one month however, cumulative mortality had become significantly lower ($P < 0.001$) for UCG 0.01 and UCG 0.05 than in the control group (41.6, 59.6 and 78.7%, respectively; Fig. 1). Cumulative mortality in the control was nonetheless still significantly lower ($P < 0.001$) than for UCG 0.10 and UCG 0.50 (91.2 and 99.6%, respectively; Fig. 1).

By the end of the experiment after 120 days, the lowest larval mortality occurred for UCG 0.01 and UCG 0.05 (58.9 and 62.7%, respectively; $P < 0.001$), while the highest mortality occurred for 0.50 UCG (99.7%; $P < 0.001$; Fig. 1). The mortality for UCG 0.10 was also very high (90.8%), and significantly lower than UCG 0.50 ($P < 0.001$). At a concentration of 0.50 g/ml, the UCG were highly effective as a larval control agent, as 97.0% of all larvae were dead by day 10 (Fig. 1). Interestingly, the mortality in the controls, which was the lowest in the beginning, steadily increased to 85.9% by day 120, which was not significantly different from that for UCG 0.10 ($P = 0.314$).

The overall mortality rate for the trough water treatment (83.7%) was higher than that in the manure solution (79.1%; $P = 0.015$). However, larval response varied considerably throughout the experiment, in particular for the control replicates (Fig. 2). In the latter, mortality in trough water was not only significantly lower than in manure solution ($P < 0.001$) but the larval population crashed somewhat latter (Fig. 2). In manure solution, overall larval mortality reached the 50% mark after 10 days, while in trough water it only occurred after c.23 days ($P < 0.001$).

Overall, there was also a significantly higher mortality rate throughout the experiment among 1st/2nd instars than 3rd/4th instars ($P < 0.001$; Fig. 3), which at the end were 86.5% and 72.3%, respectively. This differential mortality was highest for UCG 0.01, under which c.62% of the larger instars successfully hatched into adults in comparison to c.22% for 1st/2nd instars ($P < 0.001$; Fig. 10.3). Also striking was the difference between the controls, as in the latter

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group only 1.3% of larvae hatched in comparison to 27.1% for $3^{rd}/4^{th}$ instars ($P < 0.001$; Fig. 3).

Regarding the overall sex ratio, only two specimens (males) ecloded in UCG 0.50, but the percentage of adult females for UCG 0.01, UCG 0.05, UCG 0.10, and the control was 42.4, 50.1, 30.4 and 41.4%, respectively (not significantly different, $P = 0.139$). There was however, a very significant difference amongst the two instars groups ($P < 0.001$), as the percentage of females was much lower among $1^{st}/2^{nd}$ instars (25.7%) than $3^{rd}/4^{th}$ instars (56.5%). A closer look at the former group indicated a significant reduction in the percentage of females for all concentrations of UCG. This effect however, did not differ between the treatment concentrations ($P = 0.662$), suggesting that caffeine even at very low levels was capable of reducing the percentage of females in first and second instars.

DISCUSSION

Unfortunately, the mortality in the controls was excessive and compromised the overall accuracy of the results. As Figure 1 indicated, after 15 days the mortality in the control was already too high. Translocation shock might have been a factor, as shown by the differential mortality in the controls between the trough water (original larval habitat) and manure solution treatments (Fig. 2). To control for this problem, the experiment could for instance, have started from an identical batch of eggs instead of being initiated at the larval stage.

However, nutrient depletion was most likely the main cause of elevated larval mortality, especially when the experiment by Laranja et al. (2003) on *Ae. aegypti* is considered. Part of Laranja et al.'s (2003) work assessed the proportion of larvae that successfully hatched into adults, in treatments with and without added fish food. In the latter, only 5% of larvae hatched into adults in the clean water control, while the rate for UCG 0.025 was 38%. In contrast, with added food the rearing success rates were 64% and 25%, respectively. A similar outcome would have been probably obtained in this experiment, if an adequate supply of food had been given to the larvae. Although the manure solution adopted led to high larval yields in the field (e.g. Derraik & Slaney 2005), under laboratory conditions (i.e. without the likely nutrient input from the surrounding environment) it was a poor medium for mosquito larval development. In comparison for example, a laboratory work with *Oc. notoscriptus* larvae fed with fish food in controlled conditions obtained a mean rearing success rate of 91% over 13 generations (Watson et al. 2000).

Note also that the significant decrease in the percentage of adult females (down to

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c.25%) among $1^{st}/2^{nd}$ instars cannot be assessed, as the high mortality in the controls invalidated any conclusions regarding sex ratio. A baseline ratio could not be obtained, which is necessary as the sex ratio of hatching cohorts may change seasonally.

Despite the experimental problems, the results of this study are discussed due to their potential relevance to public health as, based on the data for the first 10 days of the experiment (prior to high mortality in the controls), there was very strong indication that UCG at high concentrations induced very large and relatively fast mortality of *Oc. notoscriptus* larvae. Larval mortality was initially very high for UCG 0.10, but it was not significantly different from the controls after 120 days. However, UCG 0.50 showed high toxicity to larvae, killing 97.0% in the first 10 days.

Caffeine was most likely the compound toxic to *Oc. notoscriptus* larvae, and the relatively sudden mortality could be explained by the compound's high water solubility (Gardinali & Zhao 2002). At the beginning of the experiment, there were c.1.5 mg of caffeine per gram of UCG. Tests done for UCG 0.50 samples indicated that after 5 and 10 days there were, respectively, 0.20 and 0.25 mg/ml of caffeine in solution. Thus, approximately 33% of the available caffeine in the grounds was dissolved in the above solution after 10 days. The UCG 0.50 was equivalent to approximately 0.25 mg/ml of caffeine, and Laranja et al. (2003) also obtained c.100% larval mortality with caffeine at 0.50 mg/ml. In the latter study, the development of *Ae. aegypti* larvae (a "clean water" species) was hindered at UCG 0.025, in which 75% of larvae failed to hatch, compared to 36% in the controls.

The indication that UCG could be effective as a mosquito larvicide against *Oc. notoscriptus* is of great interest. This species has a high tolerance to putrid and contaminated water, and the dosages toxic to *Oc. notoscriptus* are therefore likely to work against most other species. Further laboratory experiments and field trials are necessary to establish the actual efficacy of UCG as a larval control technique.

Although the use of pure caffeine as a mosquito larvicide is a possibility, unlike UCG, it would not be a larvicide accessible to the general public. In New Zealand, UCG could indeed be useful to control mosquito larvae of *Oc. notoscriptus* for instance, in planter bases and other containers that cannot be tipped upside down. In tropical countries such as Brazil, where the consumption of coffee is widespread, UCG may therefore constitute a cost-free (the grounds are discarded by the general population) and environmentally sound alternative to less desirable insecticides.

However, the survivorship of larvae for UCG 0.01 and UCG 0.05 in comparison to the

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controls suggested that in nutrient-poor systems, UCG in low concentrations could benefit the larvae and boost their survivorship and hence, increase the number of larvae that successfully hatch into adults. Small amounts of UCG could enhance bacterial and algal growth, thus providing food for mosquito larvae. As mentioned above, Laranja et al. (2003) obtained similar results in treatments with no added fish food, and the authors suggested that the presence of amino acids and fatty acids among other nutrients in UCG may 'feed' the larvae, and consequently overcome, at least partially, the harmful effects. As a result, the actual effects of UCG for larval control in the field are not yet known. It may be necessary to regularly add UCG to containers exposed to rainfall, as it is possible that if significant dilution occurs, the resulting low concentration of UCG may aid larval survivorship, which could be a drawback to its wider use as a larvicide. As a result, based on our results and those obtained by Laranja et al. (2003) we believe that caution is necessary, and that extensive field trials should be carried out before advocating the use of UCG as a mosquito larvae control method.

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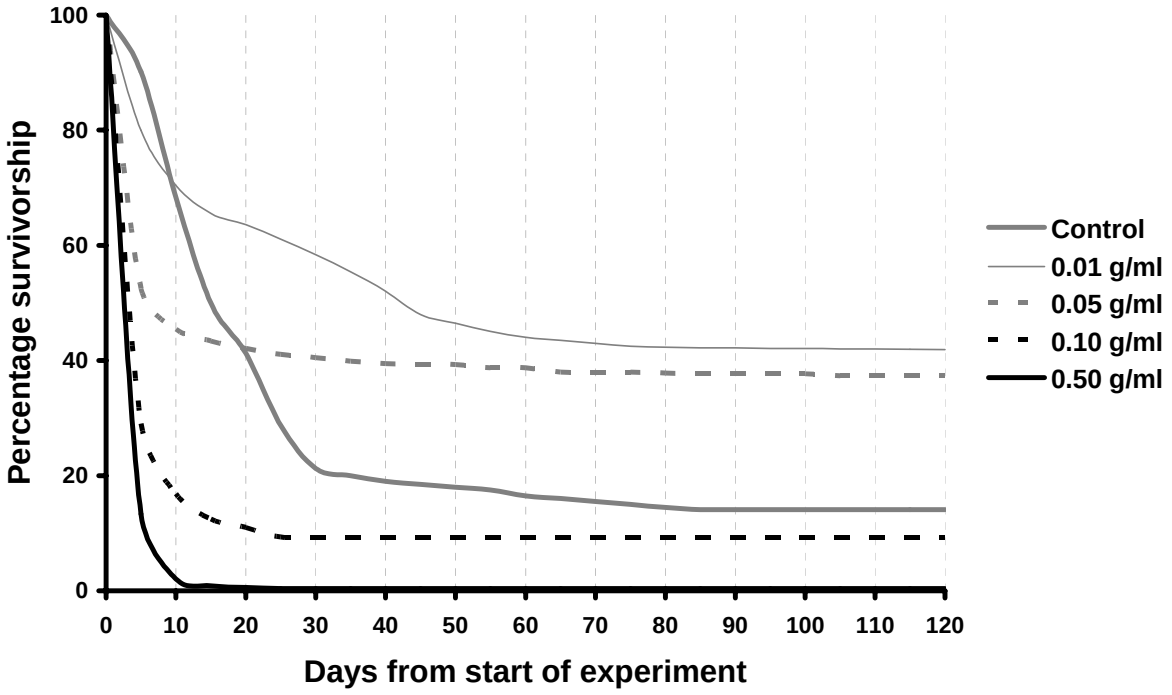

Fig. 1: Survivorship curves for *Ochlerotatus notoscriptus* larvae in the control and four different concentrations of used coffee grounds (n = 720 each), for all combined treatments.

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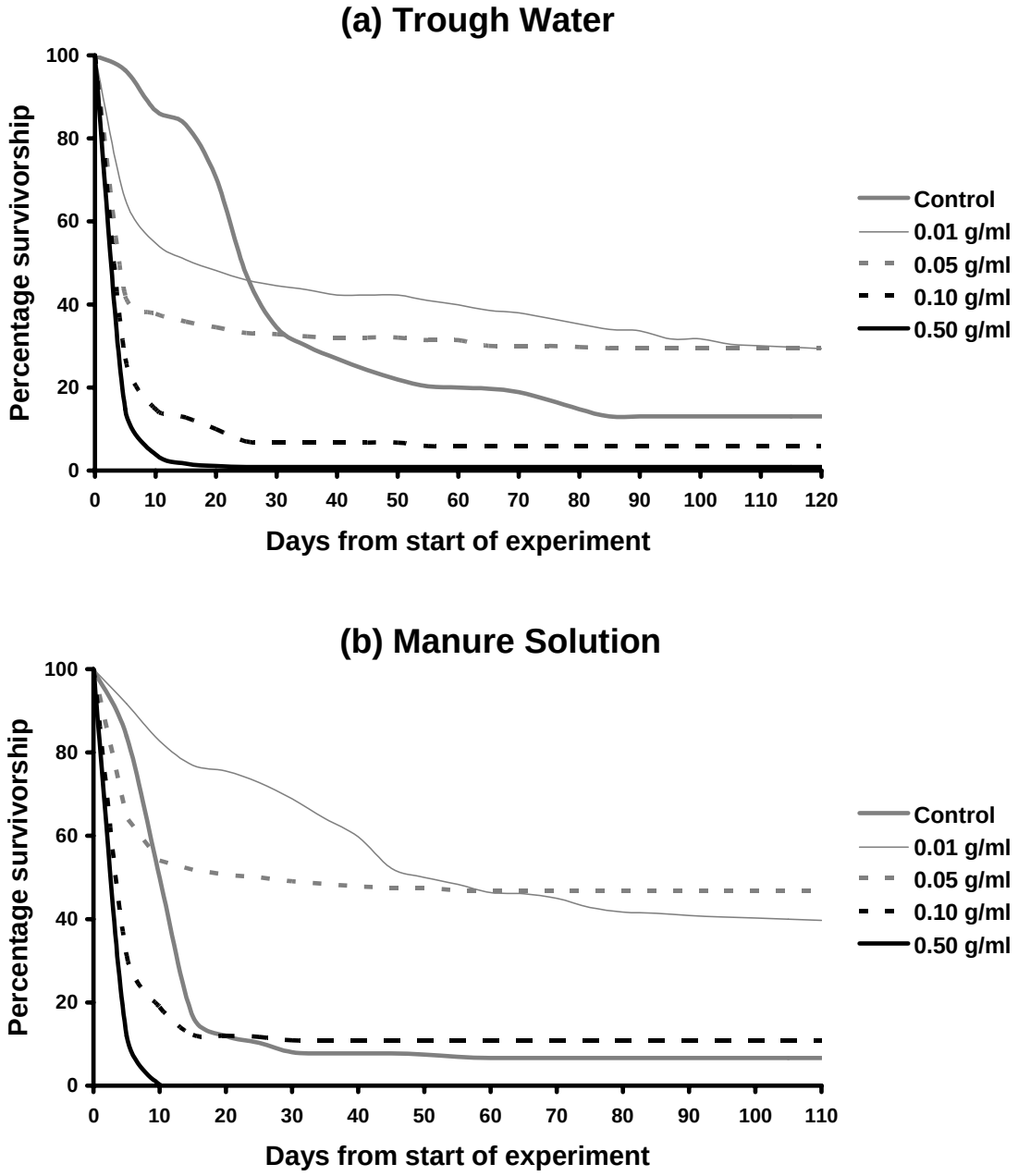

Fig. 2: Survivorship curves for *Ochlerotatus notoscriptus* larvae subjected to different treatments within (a) trough water and (b) manure solution. Note that n = 360 for each plotted treatment.

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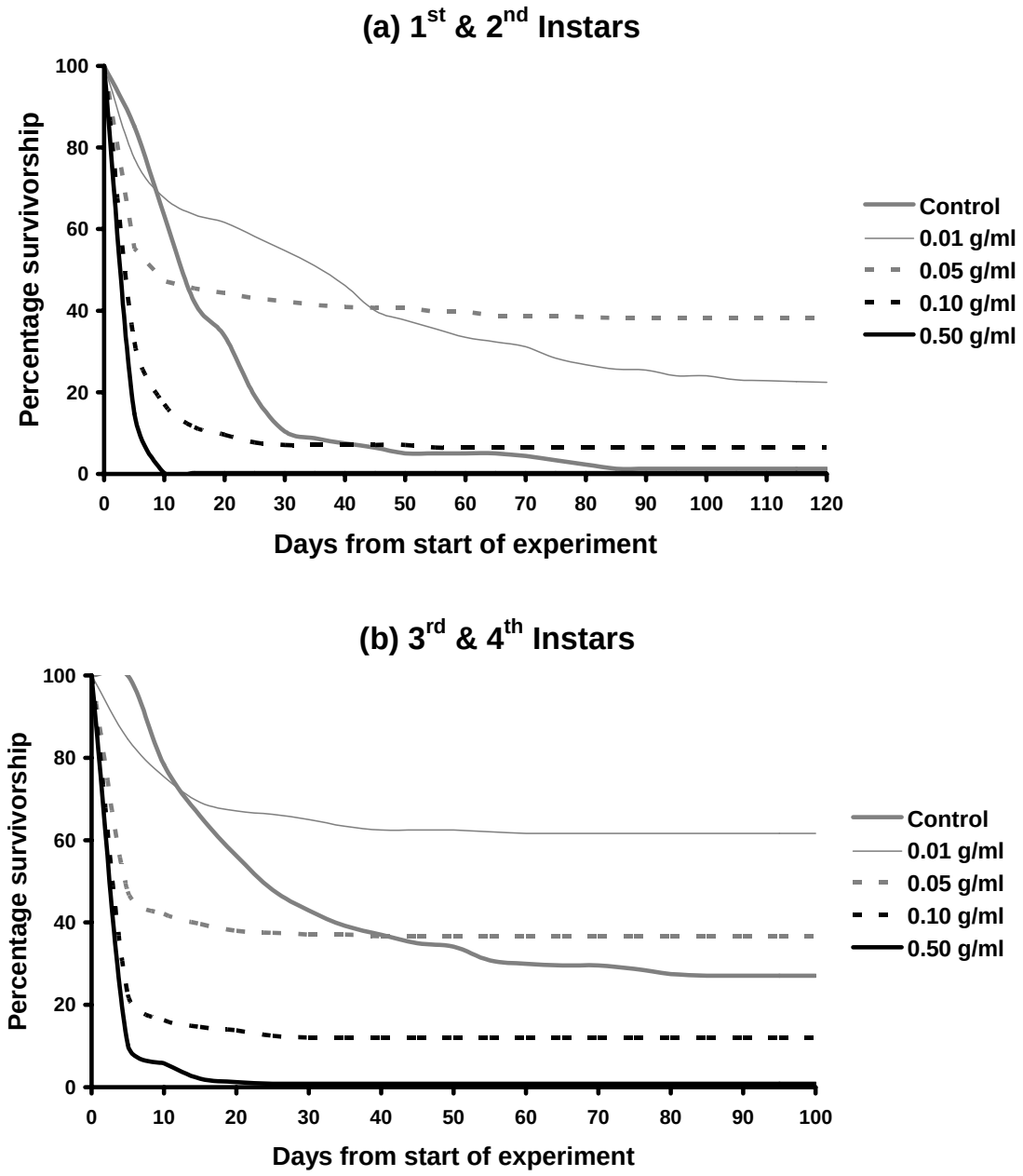

Fig. 3: Survivorship curves for *Ochlerotatus notoscriptus* larval groups subjected to different used coffee ground treatments: (a) 1st & 2nd Instars and (b) 3rd & 4th Instars. Note that n = 240 and n = 120 for the larval groups, respectively, for each plotted treatment.

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