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EFFECTS OF TWO COMMONLY USED FUNGICIDES ON THE AMPHIPOD *AUSTROCHILTONIA SUBTENUIS*

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Abstract

Fungicides are used widely in agriculture and have been detected in adjacent rivers and wetlands. However, relatively little is known about the potential effects of fungicides on aquatic organisms. The present study investigated the effects of 2 commonly used fungicides, the boscalid fungicide Filan® and the myclobutanil fungicide Systhane™ 400 WP, on life history traits (survival, growth, and reproduction) and energy reserves (lipid, protein, and glycogen content) of the amphipod *Austrochiltonia subtenuis* under laboratory conditions, at concentrations detected in aquatic environments. Amphipods were exposed to 3 concentrations of Filan (1 µg active ingredient [a.i.]/L, 10 µg a.i./L, and 40 µg a.i./L) and Systhane (0.3 µg a.i./L, 3 µg a.i./L, and 30 µg a.i./L) over 56 d. Both fungicides had similar effects on the amphipod at the organism level. Reproduction was the most sensitive endpoint, with offspring produced in controls but none produced in any of the fungicide treatments, and total numbers of gravid females in all fungicide treatments were reduced by up to 95%. Female amphipods were

more sensitive than males in terms of growth. Systhane had significant effects on survival at all concentrations, whereas significant effects of Filan on survival were observed only at 10 µg a.i./L and 40 µg a.i./L. The effects of fungicides on energy reserves of the female amphipod were different. Filan significantly reduced amphipod protein content, whereas Systhane significantly reduced the lipid content. The present study demonstrates wide-ranging effects of 2 common fungicides on an ecologically important species that has a key role in trophic transfer and nutrient recycling in aquatic environments. These results emphasize the importance of considering the long-term effects of fungicides in the risk assessment of aquatic ecosystems.

Keywords: Fungicide, Aquatic invertebrates, Reproductive toxicity, Chronic effects, Energy reserves

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INTRODUCTION

Fungicides have been used widely to control fungal diseases in agriculture, industry, home, and garden, with the predominant use being in agriculture [1,2]. After application, fungicides can be transported to nearby water bodies through spray drift, runoff, and infiltration to groundwater [3]. Fungicides have been found in surface water and sediments [4–7] at concentrations that may cause adverse effects on aquatic organisms [8–10].

The degradation of fungicides in aquatic environments varies from a few days (i.e., propiconazole) to a few months (i.e., pyrimethanil) [11] or even a few years (i.e., boscalid) [12]. Moreover, fungicides are often reapplied during growing seasons, in some cases up to 10 times

per season [6]. Therefore, organisms in adjacent streams may be exposed to fungicides for extended periods. In the literature, there are many studies on acute toxicity of fungicides to aquatic animals but very few studies on chronic toxicity [10], even though chronic toxicity data are essential for the ecotoxicological assessment of chemicals [13]. A variety of fungicides occur in the field at concentrations below those known to cause acute toxicity [4,6], but it is unclear whether they have chronic effects.

Although fungicides are designed to target fungal pathogens, their modes of action may be deleterious to nonpathogenic fungi and nontarget organisms [14]. Thus, there has been an increased interest in the effects of fungicides on aquatic leaf-decomposing fungi and leaf-shredding invertebrates, especially amphipods [14–18], because of their critical role in leaf litter decomposition, a fundamental ecosystem process in streams. Amphipods not only are key shredders of leaf litter breakdown [19] but also are central in aquatic food webs [20] and are food sources for many fish species [21]. They are sensitive to a wide range of toxicants and have been used extensively in ecotoxicology assessment [22]. Several studies have shown the adverse effects of fungicides on freshwater amphipods. For example, the feeding rate of *Gammarus fossarum* was significantly reduced after a 7-d exposure to 600 µg/L of the fungicide tebuconazole [15]. Growth and assimilation were also significantly reduced after a 3-wk exposure to the same concentration. A tebuconazole concentration of 65 µg/L significantly reduced lipid content in *G. fossarum* by approximately 20% [16]. Shredding activity of the amphipod *Gammarus pulex* was significantly reduced at 50 µg/L when applied in a mixture with the insecticide alpha-cypermethrin (0.1 µg/L and 1 µg/L) [17]. Jubeaux et al. [23] observed a significant increase in induction of vitellogenin-like proteins in *G. fossarum* when exposed to 0.1 µg/L of propiconazole.

Most studies on effects of fungicides on shredding invertebrates have been conducted in Europe using European indigenous species such as *G. fossarum* and *G. pulex*, [15,16,18,24,25], while data are lacking in some other geographical regions. However, the current world trend is to assess the response of native species to toxicants of concern because they are adapted to the local environmental conditions and can thus provide much more representative outcomes than those obtained with a foreign species [22].

Austrochiltonia subtenuis is an Australian indigenous amphipod. It is widespread in southern Australia [26,27] and is one of the most abundant species in lowland standing waters in western Victoria [28,29]. As a shredder, it has a vital role in the decomposition of organic matter in streams [30]. It is also an important food source for many fish species [31–34]. Despite its ecologically important roles in aquatic ecosystems, little is known about effects of toxicants on this species. The only available study of toxic effects on *A. subtenuis* showed that this species is more sensitive to cadmium than some other native freshwater invertebrates [35].

Boscalid and myclobutanil are 2 commonly used fungicides in Australia and in many other countries to control fungal diseases in crops, fruit trees, vegetables, and so on [11]. They are considered emerging fungicides of concern because of their high or increasing global use rates, high detection frequency in surface waters, and likely persistence in the environment [12]. Boscalid and myclobutanil are fungicides commonly found in aquatic environments [4,6]. Boscalid was often detected in stream water in Western Port, Victoria, Australia, with the highest concentration recorded at 3.3 µg/L [10]. It was also commonly detected in 3 central California (USA) coastal watersheds, at concentrations as high as 36 µg/L [5]. Myclobutanil was the most frequently detected fungicide (38% frequency of detection) in surface water of streams adjacent to a horticultural production area in southeastern Australia, with the highest concentration

reported at 2.9 µg/L [4]. In the Kisco River (NY, USA), myclobutanil was detected in 73% of water samples, with the highest concentration at 0.75 µg/L [36].

The present study investigated the effects of environmentally relevant concentrations of boscalid and myclobutanil on life history traits (survival, growth, and reproduction) and energy reserves (lipid, protein, and glycogen content) of the amphipod *A. subtenuis* under laboratory conditions. Potential indirect effects of these fungicides on the amphipod were also evaluated through assessing microbial activity on conditioned leaves that were used as a food source for the amphipods, as recent studies have shown that fungicides could alter the leaf-associated fungal community and thus have indirect effects on amphipods [24,37,38].

MATERIALS AND METHODS

Chemicals

Boscalid was applied using the commercially available product Filan® fungicide (Nufarm, Australia), containing 500 g active ingredient (a.i.)/kg. Mycobutanil was applied using the commercially available product Systhane™ 400 WP fungicide (Dow AgroSciences, Australia), containing 400 g a.i./kg. Filan and Systhane 400 WP were dissolved in deionized water to make a stock solution with a nominal concentration of 50 mg a.i./L. The stock solution was then diluted to achieve nominal boscalid concentrations of 1 µg a.i./L, 10 µg a.i./L, and 40 µg a.i./L and mycobutanil concentrations of 0.3 µg a.i./L, 3 µg a.i./L, and 30 µg a.i./L. The concentrations of boscalid and myclobutanil were determined based on the concentrations detected in the natural environment [4,5,39]. Both stock and test medium were prepared immediately prior to the initiation of testing and each water change. Water samples were collected before the experiment and sent to Advanced Analytical Australia (North Ryde, New South Wales, Australia) for analysis of boscalid and myclobutanil concentrations by liquid

chromatography–tandem mass spectrometry (LC–MS/MS). Measured and nominal concentrations were in good agreement (Supplemental Data, Table S1). In the present study, reported concentrations are nominal concentrations.

Preconditioned leaves

Green hazel (*Pomaderris aspera*) leaves were picked from trees in the Royal Botanic Gardens, Melbourne, Victoria, Australia. Leaves were cut into leaf discs (diameter, 1.3 cm) by hole punch, air-dried, and stored at room temperature until use. Before the experiment, leaf discs were preconditioned for 2 wk in a 2-L beaker containing 1 L of nutrient-enriched stream water (5 mg P as K_2HPO_4, 20 mg N as $[NH_4]_2SO_4$) [40]. This process was carried out weekly to provide freshly preconditioned leaves for the amphipods throughout the present study.

Test species

The water and *A. subtenuis* used in the experiments were collected from a nonpolluted stream, Deep Creek, Victoria, Australia. In the laboratory, the amphipods were maintained under the following experimental conditions: temperature of 21 ± 1 °C and a 16:8-h light:dark photoperiod in 5-L glass aquaria with site water under constant aeration. Organisms were fed ad libitum with preconditioned hazel leaves. To obtain juveniles for the experiment, gravid females were separated after 2 wk of acclimatization into clean 2-L glass beakers. After 1 wk, amphipods were sieved through 200-µm and 500-µm mesh sieves. The resulting juveniles that were retained on the 200-µm sieve after passing through the 500-µm sieve were transferred to new 2-L glass beakers and maintained as described. After 1 wk, juvenile amphipods that were less than 2 wk old were used in the experiment. The collected site water was kept at 4 °C and brought to room temperature prior to use in the experiment.

Experimental setup

Forty *A. subtenuis* individuals were placed randomly in 600-mL glass beakers containing 400 mL aerated stream water with the respective Filan fungicide and Systhane fungicide concentrations, with site water as controls. Each beaker contained 3 leaf discs as a food source and a 5-cm × 5-cm presoaked cotton gauze as a substrate for the amphipods. All treatments and the control had 6 replicates. The experiment was run for 56 d using the same conditions as described above in the *Test species* section. Every week, surviving amphipods were transferred gently by plastic pipette to new test medium with fresh preconditioned leaves.

At the end of the experiment, the surviving adults and produced juveniles were collected, and the number of survivors was recorded. Juveniles were produced only in the last week of the experiment. Three nongravid females were randomly selected and frozen at –20 °C for lipid, glycogen, and protein content analysis, and analyses were carried out within 2 wk. The remaining surviving adults were preserved in 70% ethanol for further examination using a Leica MS5 microscope with an ocular micrometer. Specimens preserved in ethanol were sexed, and head length was measured (from the rostrum tip to the posterior margin of the head) [41] to determine growth based on the final size, with the assumption that the mean size of amphipods per replicate was the same at the beginning of the experiment, as the amphipods were the same age. The number of gravid females and the number of embryos produced per gravid female were recorded.

A second experiment was set up without the amphipods to assess the effects of fungicides on microbial activity on fungicide-exposed leaves. Two preconditioned leaf discs were exposed to the same nominal fungicide concentrations as the ones in the main experiment. There were 6 replicates for each treatment. After 1 wk, microbial respiration on hazel leaf discs was measured using changes in oxygen concentration following the method described by Carlisle and Clements

[42]. Dissolved oxygen was measured with a water quality meter (smartCHEM-LAB, TPS, Australia) at the beginning and end of a 24-h incubation period. Subsequently, leaf discs were dried in an oven for 24 h at 60 °C to calculate the microbial leaf mass loss based on the mean dry weight of 30 randomly chosen preconditioned leaf discs at the start of the experiment (11.91 ± 0.38 mg, mean ± standard error).

Determination of lipid, glycogen, and protein content

The lipid, glycogen, and protein assays were carried out using a Synergy 2 microplate reader (Biotek Instruments). Lipid and glycogen content were measured following the method described by Van Handel [43,44], using commercial vegetable oil and glucose as the standards but modified for the use of a microplate reader. The volume of solution in each well was 60 µL; absorbance is measured at 490 nm for lipid and 625 nm for glycogen. Protein content was determined using a modified Lowry assay (Bio-Rad DC method), with bovine serum albumin as the standard [45].

Statistical analysis

Treatment effects on survival, growth, reproduction, energy reserves, and microbial activity were analyzed using a one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test; separate analyses were done for each fungicide. All data were checked for normality using the Shapiro–Wilk test and homogeneity of variance using Levene's test. If the normality assumption was violated, data were analyzed by the Kruskal–Wallis test followed by the Dunn–Bonferroni post hoc test. If the assumption of homogeneity of variance was not met, data were analyzed by Welch's *F* test followed by the Games–Howell post hoc test. If the results of the overall ANOVA showed no significant difference, then linear contrast analyses were performed to determine whether any relationship existed between the measured endpoint and

fungicide concentrations. Statistical analysis was performed using SPSS Version 23 (IBM).

RESULTS AND DISCUSSION

Survival

Survival of *A. subtenuis* was affected by Filan and Systhane (Figure 1) and decreased with increasing fungicide concentrations ($F_{(1,20)} = 28.59$, $p < 0.001$, and $F_{(1,20)} = 17.63$, $p < 0.001$, respectively). There was a statistically significant difference in survival among Filan treatments ($\chi 2_{(3)} = 13.514$, $p = 0.004$) with significantly lower survival at 10 µg a.i./L and 40 µg a.i./L ($p = 0.007$ and $p = 0.015$, respectively) but no difference at 1 µg a.i./L ($p = 0.667$) compared with the control. Systhane exposure also yielded a significant difference in survival among treatments ($F_{(3,20)} = 10.308$, $p < 0.001$). Amphipods from all 3 Systhane treatments ($p < 0.05$) had significantly lower survival compared with the control. Available chronic toxicities of boscalid and myclobutanil (21-d no-observed-effect concentration [NOEC]) on *Daphnia magna* were similar, at 1 mg/L and 1.3 mg/L, respectively [11], and much lower compared with the chronic toxicity of these fungicides on *A. subtenuis* as observed in the present study. Increasing toxicity of boscalid and myclobutanil in *A. subtenuis* compared with *D. magna* could be explained by increasing the exposure period in the present study, which was 56 d compared with 21 d. During the long-term exposure period, amphipod survival in fungicide treatments gradually decreased over time (Supplemental Data, Figure S1). This is in agreement with results observed for other fungicides, such as tebuconazole; Sancho et al. [13] found that survivorship of *D. magna* did not decrease after exposure to 1.14 mg/L tebuconazole over 14 d but was significantly reduced by 6% compared with controls after 21 d. Species sensitivity could also be a reason for the different toxic effects between studies. The amphipod *Hyalella azteca*, for example, was also reported to be more sensitive to the fungicide trifloxystrobin (96-h median lethal concentration [LC50] =

24.7 µg/L) [46] than *D. magna* (96-h LC50 = 530 µg/L) [8].

Growth

Fungicide exposure also resulted in smaller head lengths, but significant effects were observed only in females (Figure 2). Females were smaller than males in general, and the head length was reduced up to 7% in both Filan and Systhane treatments compared with the control. Other studies have also shown decreased growth of aquatic organisms after fungicide exposure [13,15,47]. A constant exposure to Filan and Systhane over a long time may reduce the amphipods' ability to acquire adequate nutrition for growth [13] or increased consumption of the energy reserves to manage toxic stress [10]. Zubrod et al. [15] reported significantly reduced growth and feeding rates of the amphipod *G. fossarum* after a 3-wk exposure to tebuconazole at 600 µg/L. The reduction in growth of the oligochaete *Tubifex tubifex* was accompanied by a reduction in protein and glycogen contents as a result of exposure to the fungicide fenhexamid for 7 d [47]. Several studies have tended to use male individuals to assess the effects of toxicants on amphipods [15,25,48]. The present study demonstrated that the female amphipods were more sensitive than the males in terms of growth and that toxic effects could be neglected if both males and females were not used in the experiment or measured independently. For instance, Zubrod et al. [16] reported substantially but not significantly reduced growth of *G. fossarum* after a 5-wk exposure to tebuconazole at 65 µg/L because of high variability. A plausible explanation for this observation is the difference in response of female and male amphipods and the fact that they were not measured separately. However, this difference is more likely happen if the amphipods are exposed to toxicants during sexual maturation when energy requirements during oogenesis and brooding in females are higher than the energy-demanding process of spermatogenesis in males. In these circumstances, females would have less energy available for

growth and to cope with toxic stress than males [10]. In the natural environment, this delay in growth of female amphipods could consequently impair the reproduction of the population, because organisms need to reach a certain size before reproduction can occur [49] and larger organisms produce larger broods than smaller individuals [50].

Reproduction

Filan and Systhane had extreme adverse effects on amphipod reproduction, with a significant decrease in number of gravid females (Figure 3), a large decrease in the number of embryos per gravid females (Figure 4), and no production of offspring in all fungicide treatments. The total number of gravid females in both fungicide treatments declined significantly by approximately 95% of controls at the highest concentrations tested. Similarly, the number of embryos per gravid female declined by approximately 87% at the highest concentrations, with effects apparent even at low concentrations.

Some studies on chronic toxicity found that mortality was a more sensitive endpoint than reproductive parameters [51,52], whereas most authors reported that reproduction was a better and more sensitive indicator of effect than survival [10,53]. The results of the present study support the latter conclusion. Even though significant effects on survival, number of gravid females, and number of embryos per gravid female occurred at the same concentrations for Filan and Systhane (10 µg a.i./L and 0.3 µg a.i./L, respectively), the magnitude of the effect was much higher in reproduction than in survival. At 10 µg a.i./L of Filan, survival was reduced by 27% of the control while the number of gravid females was reduced by 84% from the control. Similarly, at 0.3 µg a.i./L of Systhane, the reductions in survival and number of gravid females were 22% and 89%, respectively. Moreover, there was no significant effect on survival at the lowest Filan treatment, but there were significant effects on reproduction, with no released offspring. The

present results are in agreement with the study by Sancho et al. [13], who reported that *D. magna* reproduction was seriously affected by the fungicide tebuconazole, with number of neonates per female being the most sensitive parameter.

Decreased growth of female *A. subtenuis* is a clear reason for the reduction in amphipod reproductive success, as body size is a determining factor for the onset of the reproductive phase of amphipods [54]. This hypothesis was supported by France [55] and Glazier [56], who reported the size–fecundity relationship of the amphipod *H. azteca* and *Gammarus minu*. Vu et al. [10] also found that growth and reproduction of the amphipod *Allorchestes compressa* were significantly reduced after exposure to Filan for 6 wk. Decreased amphipod reproduction in the wild would eventually result in lower abundance, which would reduce amphipods' contribution to local leaf litter breakdown and also reduce the food supply for higher trophic levels [16]. Therefore, amphipod reproduction endpoints could provide important information for assessing risk of fungicides to the aquatic ecosystems.

Energy reserves

Glycogen content contributed a small amount to amphipod energetics compared with lipid and protein content and was not significantly different between treatments (Figure 5). Protein and lipid content were altered after fungicide exposure. The protein content decreased significantly with increasing Filan concentrations ($F_{(1,20)} = 11.23$, $p = 0.003$), whereas the lipid content decreased significantly with increasing Systhane ($F_{(1,20)} = 7.13$, $p = 0.015$). Although the present study analyzed the whole body contents, which may be influenced by the growth effects, our results agree with previous studies that reported lower lipid and protein content in aquatic invertebrates after exposure to fungicides [10,16,57,58]. This decrease in lipid and protein content in amphipod after fungicide exposure has been ascribed to the increased energy

expenditure to deal with toxic stress [10]. Toxicants often induce effects on different metabolic pathways, but proteins and lipids are usually easily available for energy production and are rapidly catalyzed to meet energy demands during stress periods [57]. A decrease in nutritional quality of food (leaf palatability), resulting from the exposure of fungicides, could also contribute to the decrease in energy reserves, as microbial biomass is an important food source for shredding invertebrates including amphipods [30]. Zubrod et al. [16] observed a significantly reduced lipid content of *G. fossarum* (~20%) following exposure to 65 µg/L tebuconazole, which was correlated with decreased fungal biomass and sporulation on the conditioned leaves used to feed the amphipods at 65 µg/L. The results for microbial respiration and leaf mass loss of the present study may suggest that amphipod food quality was affected; however, this conclusion needs to be treated with caution (discussed in the section **<ZAQ;1>***Microbial respiration and microbial leaf mass loss*).

Several studies have shown that energetic parameters are early indicators of life history effects in freshwater organisms exposed to fungicides [10,57,58]. However, the most sensitive energetic parameter varies among organisms and fungicides. Sancho et al. [57] and Vu et al. [10] found that lipid content was the most sensitive energetic parameter for daphnia and amphipods. In contrast, Mosleh et al. [47] and Fidder et al. [58] demonstrated that protein and glycogen content were affected in oligochaetes and gastropods after fungicide exposure. In the present study, protein content of *A. subtenuis* after Filan exposure was the most affected energetic parameter, but lipid content was most affected by Systhane exposure. The differences between the effects of Filan and Systhane on *A. subtenuis* energetics could be attributed to the difference in their modes of action. Although the modes of action of these fungicides on aquatic invertebrates are unclear, modes of fungicide action could predict analogous mechanisms of

toxicity, target sites, and toxic effects for nonfungal species, as many biochemical pathways and processes are conserved across species [59]. Filan belongs to the group of succinate dehydrogenase inhibitor fungicides, which disrupt fungal respiration, while Systhane belongs to the demethylation inhibitor group of fungicides, which inhibit sterol biosynthesis in fungal membranes [11]. Because demethylation inhibitor fungicides interact with 14-α-demethylase, an enzyme involved in the biosynthesis of cholesterol in animals, it follows that Systhane may also interrupt the lipid build-up process in *A. subtenuis* [16]. This could lead to the observed decrease in lipid content after exposure to Systhane in the present study. Our results in terms of fungicide effects on amphipod energetics further emphasize the importance of understanding fungicide modes of action on nontarget species.

Microbial respiration and microbial leaf mass loss

Microbial leaf mass loss decreased significantly with increasing Filan and Systhane concentrations (Figure 6). However, significant differences from the control were observed only in Systhane treatments, for which the leaf mass loss declined by approximately 50%. Microbial respiration also decreased in Filan and Systhane treatments ($F_{(1,17)} = 4.97$, $p = 0.04$, and $F_{(1,15)} = 7.71$, $p = 0.014$, respectively), but no significant differences from the control were observed (Supplemental Data, Figure S2). These decreases in microbial respiration and microbial leaf mass loss could indicate the reduction in food quality that subsequently has indirect effects on the amphipod. The colonization of microbial organisms on senescent leaves, commonly known as conditioning, benefits the shredders in 2 main ways: by producing enzymes that are not available in invertebrate digest system to promote the breakdown of complex structural compounds of the leaves and to transform them to simple compounds edible to invertebrates, and by providing an additional food source for invertebrates, as fungal mycelia colonizing leaves

have higher nutrient content than senescent leaves [30]. Therefore, the effects of fungicides on nontarget fungi and bacteria that have colonized the leaves could reduce the palatability of leaves and affect shredding invertebrates. Rasmussen et al. [17] found that macroinvertebrates increased their consumption of beech leaves (*Fagus sylvatica*) with lower microbial biomass to compensate for the reduced nutritional quality of this leaf litter after only a 3-h exposure to the fungicide propiconazole at 50 µg/L and 500 µg/L. Zubrod et al. [38] also found that fungal species richness was significantly reduced in leaves conditioned in fungicide mixture for 12 d, and the consumption of such conditioned leaves over 24 d resulted in a significantly reduced *G. fossarum* growth (~40%). Effects of fungicides on invertebrate feeding behavior [17] or amphipod growth [38] could be the result of either a toxic effect of fungicides accumulated in leaf material or a reduction in food quality through fungicide-induced changes in microbial composition [60]. However, Flores et al. [37] reported that imazalil fungicide (ranging from 0.1 µg/L to 100 µg/L) significantly decreased the number of fungal species and altered the fungal sporulation rate but caused no significant effects on consumption rate of the amphipod *Echinogammarus berilloni* compared with the control. The present results may indicate effects of tested fungicides on conditioning; however, this indication must be taken with caution, because leaves were preconditioned, which may have provided sufficient time for microbial decomposers to bring about the changes in the leaf substrate required by the shredders.

CONCLUSIONS

Overall, findings from the present study showed that both Filan (boscalid) and Systhane (myclobutanil) had adverse effects on survival, growth, reproduction, and energy reserves of *A. subtenuis* at concentrations detected in natural environments. These effects could be a combination of direct fungicide toxicity on amphipods and indirect effects on food quality. This

is the first study of long-term effects of fungicides on *A. subtenuis*, an Australian native and ecologically important species. The sensitivity of *A. subtenuis* to the fungicides emphasizes the importance of developing standard toxicity bioassays for this species to be used in environmental impact assessments of fungicides as well as other toxicants in Australia. Moreover, the most significant observed effects on reproduction outputs, which are currently scarce in the literature, suggest the need for consideration of these endpoints in future studies on fungicide toxicity, as a delay in reproduction could negatively affect the viability of the population at an ecological scale.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3584.

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Data Availability—Data, associated metadata, and calculation tools are available from the corresponding author (htvu@student.unimelb.edu.au).

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Figure 1. Survival percentages (mean ± standard error) of *Austrochiltonia subtenuis* in control, Filan, and Systhane treatments after 56-d exposure (n = 6). An asterisk (*) denotes significant difference from control ($p \leq 0.05$).

Figure 2. Head length (mean ± standard error) of *Austrochiltonia subtenuis* in control, Filan, and Systhane treatments after 56-d exposure. Females are blue bars and males are red bars (n = 6). An asterisk (*) denotes significant difference from control ($p \leq 0.05$). a.i. = active ingredient.

Figure 3. Number of gravid females (mean ± standard error) of *Austrochiltonia subtenuis* in control, Filan, and Systhane treatments after 56-d exposure (n = 6). An asterisk (*) denotes significant difference from control ($p \leq 0.05$). a.i. = active ingredient.

Figure 4. Number of embryos per gravid female (mean ± standard error) of *Austrochiltonia subtenuis* in control, Filan, and Systhane treatments after 56-d exposure (n = 6). An asterisk (*) denotes significant difference from control ($p \leq 0.05$). a.i. = active ingredient.

Figure 5. Concentrations of lipid (blue bars), glycogen (red bars), and protein (green bars) (mean ± standard error) of *Austrochiltonia subtenuis* in control, Filan, and Systhane treatments after 56-d exposure (n = 6). An asterisk (*) denotes significant difference from control ($p \leq 0.05$). a.i. = active ingredient.

Figure 6. Hazel leaf mass loss (mean ± standard error) in control, Filan, and Systhane treatments after 7-d exposure (n = 6). An asterisk (*) denotes significant difference from control ($p \leq 0.05$). a.i. = active ingredient.

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