

# Insecticides reduce survival and the expression of traits associated with carnivory of carnivorous plants

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**Abstract** While agrochemical pollution is thought to be an important conservation threat to carnivorous plants, the effects of insecticides on these taxa have not been quantified previously. Using a combination of lab- and field-based experiments, we tested the effects of commercial and technical grades of three widely used insecticides (carbaryl, lambda-cyhalothrin, and malathion) on survival and the expression of traits associated with carnivory of pink sundews (*Drosera capillaris*) and Venus flytraps (*Dionaea muscipula*). Commercial grades were generally more harmful than technical grades under lab and field conditions, but all three insecticides were capable of reducing both survival and the expression of traits associated with carnivory within recommended application rates. However, pink sundews appeared to be more susceptible to insecticides than Venus flytraps, perhaps because of larger numbers of digestive glands on the leaf surfaces. We make several recommendations for future research directions, such as examining the long-term effects of insecticides on carnivorous plant populations, for example in terms of growth rates and fitness. Additionally, future research should include representative species from a wider-range of carnivorous plant growth forms, and explore the mechanism by which insecticides are harming the plants. Given the effects we observed in the present study, we suggest

that the use of insecticides should be carefully managed in areas containing vulnerable carnivorous plant species.

**Keywords** Carnivorous plants · Conservation · *Dionaea muscipula* · *Drosera capillaris* · Insecticides

## Introduction

Pollution is thought to be one of the main causes of species declines in the United States (Wilcove and Master 2005). Agrochemicals, in particular, are a widespread source of pollution, whether through direct application of fertilizers, herbicides, and insecticides, or through run-off and drift. While many studies have examined the indirect effects of agrochemicals on non-target organisms (Rohr et al. 2006; Desneux et al. 2007), to the best of our knowledge, only one previous study has quantitatively examined the effects of any type of pesticide on carnivorous plants (Smith and Pullman (1997) examined the effects of an aquatic herbicide on a *Utricularia* sp., among other freshwater plants). This is surprising given that pollution is often cited as a threat to carnivorous plants (Folkerts 1977, 1990; Jennings and Rohr 2011), ostensibly because these plants are commonly found in wetland areas which frequently accumulate agrochemicals (Clark et al. 1993; Davis and Froend 1999).

Among the main groups of agrochemicals, herbicides would likely be considered to pose the greatest threat to non-target plants. However, some insecticides also are known to be highly phytotoxic and capable of exerting direct negative effects on plants (Murthy and Raghu 1990; Gange et al. 1992; Peterson et al. 1994; Straw et al. 1996). Furthermore, insecticides could exert important indirect negative effects for carnivorous plants in particular, such as a reduction in the abundance of potential prey. Carbaryl,

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lambda-cyhalothrin, and malathion are three widely used insecticides in the United States (Kiely et al. 2004). Each is used to control adult or larval mosquitoes (Milam et al. 2000; Suwanchaichinda and Brattsten 2001; Lawler et al. 2007) and thus they are regularly applied either directly on or near wetlands that might contain carnivorous plants. Using a combination of lab- and field-based experiments, we quantified the effects of both commercial and technical grades of these insecticides on survival and the expression of traits associated with carnivory of two carnivorous plant species: pink sundews (*Drosera capillaris*) and Venus flytraps (*Dionaea muscipula*). Pink sundews capture insect prey with sticky mucilage secreted from modified leaf trichomes, and they are commonly found in wetland habitats throughout the southeastern United States (Schnell 2002). Conversely, Venus flytraps are limited to wet pine savannas in the Carolinas and are listed as vulnerable by the International Union for the Conservation of Nature (Schnell et al. 2000; Schnell 2002), with pollution often considered a threat (Jennings and Rohr 2011).

Along with most carnivorous plant species, pink sundews and Venus flytraps have small, fragile roots (Adlassnig et al. 2005) and their leaf surfaces are covered in digestive glands (Juniper et al. 1989), both of which could increase their susceptibility to insecticides in comparison to many other plants. Consequently, our hypotheses were as follows: 1) insecticides will directly reduce the survival of carnivorous plants, and 2) given that carnivorous plants under stress often reduce their expression of traits associated with carnivory (i.e. investment in structures involved in prey capture), surviving pink sundews and Venus flytraps will produce fewer leaves with mucilage, and fewer traps respectively. Quantifying the effects of insecticides on the expression of traits associated with carnivory has important implications for carnivorous plants at the population-level, and at the broader community-level (Clements and Rohr 2009). For example, if insecticides are capable of changing the expression of these traits used by carnivorous plants for prey capture, they could be indirectly affecting the abundance of arthropods frequently utilized as prey.

## Materials and methods

Pink sundews were collected from the University of South Florida Ecological Research Area (ERA), and Venus flytraps were ordered from [www.bugbitingplants.com](http://www.bugbitingplants.com) (Venus flytraps are threatened, preventing us from studying them in or collecting them from the field). Plants in all lab experiments were maintained at 23°C under full spectrum lighting (14L/10D) and covered with Plexiglas to maintain humidity. Each experiment (lab- and field-based) ran for 4 weeks, and survival (plants were considered dead when

all structures had turned black) and the expression of traits associated with carnivory (the number of leaves with mucilage and traps for pink sundews and Venus flytraps, respectively) were quantified on a weekly basis.

### Experiment i: effects of commercial grade insecticides on pink sundews in the lab

Seventy-two pink sundews (mean diameter  $2.28 \pm 0.59$  cm) were planted in individual 9 cm diameter plastic cups filled to 5 cm with sand, as often they are locally found in extremely sandy soils. Treatments consisted of a de-ionized (DI) water control and commercial grades of the insecticides carbaryl (GardenTech® Sevin®), lambda-cyhalothrin (Spectracide® Triazicide®), and malathion (Spectracide®), with 18 replicates of each of the four treatments. At the start of the experiment, 5 ml of the appropriate insecticide was applied directly to the sundews using a spray bottle and following the recommended application instructions on each product. This resulted in nominal application rates for active ingredient (a.i.) of 9.91 kg/ha (0.126%) for carbaryl, 0.16 kg/ha (0.002%) for lambda-cyhalothrin, and 5.19 kg/ha (0.003%) for malathion (for comparative purposes, percentages of a.i. per 5 ml are provided in parentheses throughout). The rates of insecticide application used for this experiment represented an overspray scenario for mosquito control, but were comparable to the rates used for control of agricultural pests.

### Experiment ii: dose–response of pink sundews to technical grade insecticides in the lab

To each of five randomly chosen sundews, we applied 99.1 (1.26%), 9.91 (0.126%), 0.991 (0.0126%), or 0.0991 (0.00126%) kg of a.i./ha of carbaryl; 1.6 (0.02%), 0.16 (0.002%), 0.016 (0.0002%), 0.0016 (0.00002%) kg of a.i./ha of lambda-cyhalothrin; or 51.9 (0.03%), 5.19 (0.003%), 0.519 (0.0003%), 0.0519 (0.00003%) kg of a.i./ha of malathion (based on serial dilutions of the highest concentration). The rates of insecticide application used in this experiment represented a range of scenarios. The lowest rates were below those typically applied for mosquito control, while the highest rates exceeded those typically used for control of agricultural pests. All insecticides were technical grade (purities > 98%, Chemservice, PA). The highest rate of application of each chemical was one order of magnitude higher than the rates of application for the commercial forms (i.e. active ingredients) used in Experiment i. Additionally, we had five replicates of both DI water and acetone (10%, solvent used to get chemicals in solution) controls, resulting in 14 total treatments and 70 individual plants (mean diameter  $2.13 \pm 0.39$  cm). Sundews were planted in individual

9 cm diameter plastic cups filled to 5 cm with sand, and at the start of the experiment, 5 ml of the appropriate insecticide was applied directly to the sundews using pipettes. Pipettes were used in this experiment for logistical purposes, and we are confident that similar amounts of insecticides reached both the plants and the substrate compared to the spray bottles in the other experiments.

#### Experiment iii: effects of commercial and technical grade insecticides on pink sundews in the field

To determine the ecological relevance of Experiments i and ii, we conducted a field experiment. We established 32 10- × 10-cm plots at the ERA, with each plot containing three sundews (mean diameter  $5.07 \pm 1.38$  cm). To each of four randomly chosen plots, we applied one of eight treatments: DI water, acetone (10%), or commercial or technical forms of carbaryl, lambda-cyhalothrin, or malathion. At the start of the experiment 5 ml of the appropriate insecticide was applied directly to the sundews using spray bottles, and there were two subsequent applications after 10 and 20 days (within the range of recommended application frequency). The field plots were slightly larger in area than the plastic cups used in the lab, and our field rates of application for insecticide a.i./plot were: 6.3 kg/ha (0.126%) for carbaryl, 0.1 kg/ha (0.002%) for lambda-cyhalothrin, and 3.3 kg/ha (0.003%) for malathion. The rates of insecticide application used for this experiment represented an overspray scenario for mosquito control, but were comparable to the rates used for control of agricultural pests.

#### Experiment iv: effects of commercial and technical grade insecticides on Venus flytraps in the lab

Sixty-four 2-year old Venus flytraps (mean fresh-weight  $1.03 \pm 0.41$  g) were planted in individual 9 cm diameter plastic cups filled to 5 cm with a mixture of 2/3 peat moss and 1/3 perlite. We used plants of the same age and similar fresh-weight to minimize variation in trap size. To each of eight randomly chosen plants we then applied 5 ml of one of the following treatments using spray bottles: DI water, acetone (10%), or commercial or technical forms of carbaryl, lambda-cyhalothrin, or malathion, using the same concentrations as in Experiment i. There were two subsequent applications of each treatment after 10 and 20 days (within the range of recommended application frequency).

#### Statistical analyses

For each experiment we first tested for differences between water and solvent (acetone) controls. No significant differences between controls were detected for any experiment

(all  $P > 0.05$ ) and, consequently, the controls were pooled together. Survival analyses were conducted using the Cox proportional hazards model (package ‘survival’, function ‘coxph’) in R 2.11.1 (R Development Core Team 2010), and for the survival dose–response in Experiment ii we compared each insecticide treatment to the controls separately. In all survival analyses, we then conducted multiple comparisons between treatments using log-likelihood ratio tests, controlling for the false discovery rate using the Benjamini-Hochberg correction (package ‘multtest’, function ‘mt.rawp2adjp’). We used analysis of covariance (ANCOVA) in Statistica 9.1 to test for the effects of treatments on the difference in the number of traps or mucilage-producing leaves (i.e. the starting number of trap of mucilage-producing leaves minus the final number) with the starting number of traps or mucilage-producing leaves as the covariate. For the dose–response in Experiment ii we compared each insecticide treatment to the controls separately as continuous predictors, and then in separate tests as categorical predictors. All multiple comparisons on the difference in the number of traps or mucilage-producing leaves were made using Dunnett’s test in Statistica 9.1.

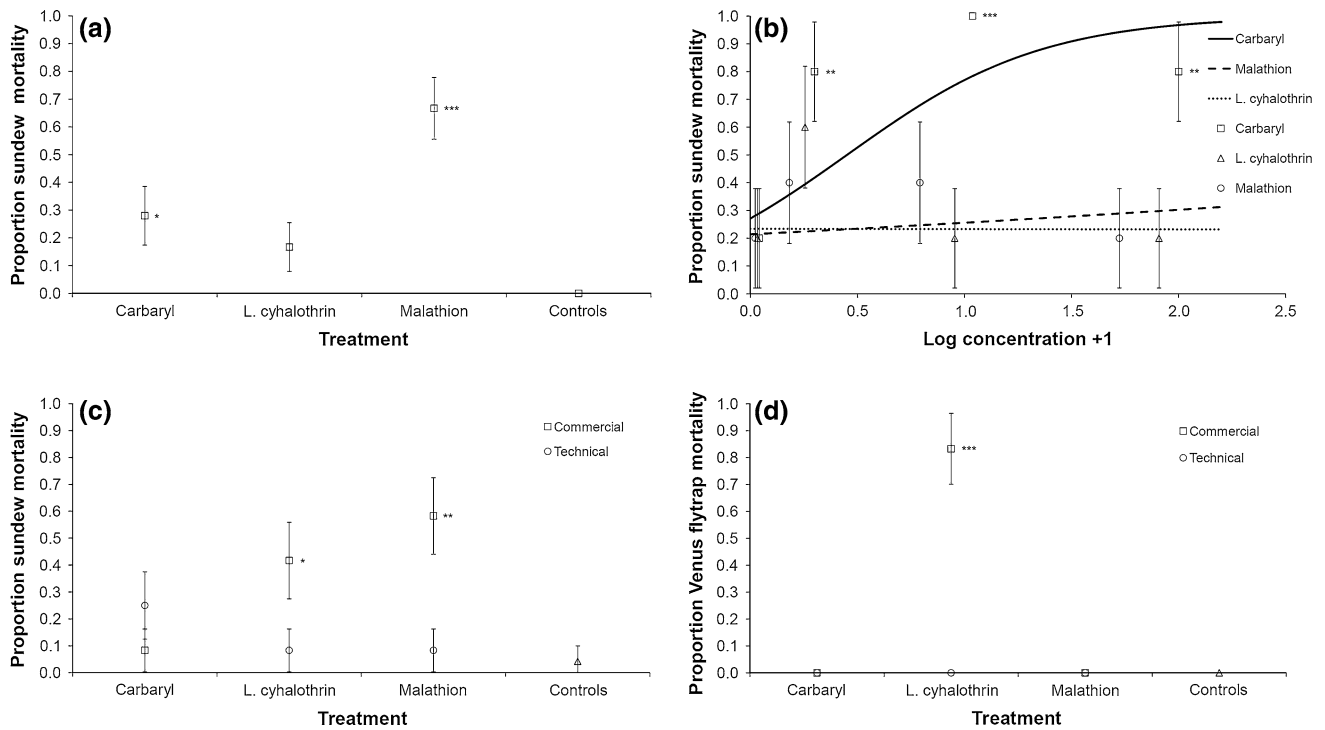
## Results

#### Experiment i: effects of commercial grade insecticides on pink sundews in the lab

We found significant effects of treatment on survival ( $\chi^2 = 18.38$ ,  $df = 3$ ,  $P < 0.001$ ) and the number of mucilage-producing leaves ( $F = 16.21$ ,  $df = 3$ ,  $67$ ,  $P < 0.001$ ). Relative to controls (0% mortality), carbaryl (28% mortality;  $P = 0.018$ ) and malathion (66.7% mortality;  $P < 0.001$ ), but not lambda-cyhalothrin (16.7% mortality;  $P = 0.056$ ), significantly reduced survival (Fig. 1a). Carbaryl, malathion, and lambda-cyhalothrin significantly reduced the number of mucilage-producing leaves (all  $P < 0.001$ ) (Table 1).

#### Experiment ii: dose–response of pink sundews to technical grade insecticides in the lab

There was a dose–response on survival of carbaryl-treated plants with all but the lowest concentration significantly reducing survival relative to controls, but there was no detected effect on survival for lambda-cyhalothrin- or malathion-treated plants (Fig. 1b). Additionally, there were significant dose-responses of carbaryl ( $F = 7.93$ ,  $df = 1$ ,  $28$ ,  $P = 0.009$ ) and lambda-cyhalothrin ( $F = 4.3$ ,  $df = 1$ ,  $28$ ,  $P = 0.048$ ) on the difference in mucilage-producing leaves, but there was no detected effect on the difference in



**Fig. 1** Proportion mortality from Experiments i (a), ii (b), iii (c), and iv (d) (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ). Points represent means and error bars are derived from proportion mortality. Curves for b represent logistic regression

**Table 1** Summary of the effects of three insecticides on carnivorous traits in pink sundews (PS) and Venus flytraps (VF)

Treatment (kg/ha)	Mean difference in mucilage-producing leaves, or traps			
	Experiment i PS (lab)	Experiment ii PS (lab)	Experiment iii PS (field)	Experiment iv VF (lab)
Carbaryl				
99.1		-4.2*** (0.84)		
9.91 (commercial)	-8.23*** (3.43)		-10.25 (3.42)	-3.38*** (3.46)
9.91 (technical)		-5.4*** (1.52)	-9.08 (2.68)	2 (1.77)
0.991		-5.2*** (1.3)		
0.0991		-2.4 (2.3)		
Lambda-cyhalothrin				
1.6		-4** (1)		
0.16 (commercial)	-8.28*** (3.74)		-7.92 (3.06)	-9.25*** (3.96)
0.16 (technical)		-3.2** (1.92)	-9.25 (2.67)	0.13 (1.89)
0.016		-2.8* (2.39)		
0.0016		-3* (2.12)		
Malathion				
51.9		-2.4 (2.7)		
5.19 (commercial)	-9.39*** (1.31)		-5.92 (3.92)	0.25 (2.25)
5.19 (technical)		-3* (3.16)	-8.25 (4.54)	0.38 (3.38)
0.519		-3.6** (2.07)		
0.0519		-2 (2)		
Controls	-1.06 (6.44)	0 (2.49)	-6.5 (3.66)	0.88 (1.36)

Shown are means (SEM) (Dunnett's test: \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ )

mucilage-producing leaves for malathion treated plants ( $F = 1.4$ ,  $df = 1, 28$ ,  $P = 0.246$ ) (Table 1).

Experiment iii: effects of commercial and technical grade insecticides on pink sundews in the field

There was a significant effect of treatment on sundew survival ( $\chi^2 = 20.02$ ,  $df = 6$ ,  $P = 0.003$ ), with the commercial grades of lambda-cyhalothrin (41.7% mortality;  $P = 0.035$ ) and malathion (58.3% mortality;  $P = 0.007$ ) significantly reducing survival relative to controls (4.2% mortality) (Fig. 1c). However, there was no significant effect of treatment on the number of mucilage-producing leaves of sundews in the field ( $F = 1.28$ ,  $df = 6, 24$ ,  $P = 0.302$ ) (Table 1).

Experiment iv: effects of commercial and technical grade insecticides on Venus flytraps in the lab

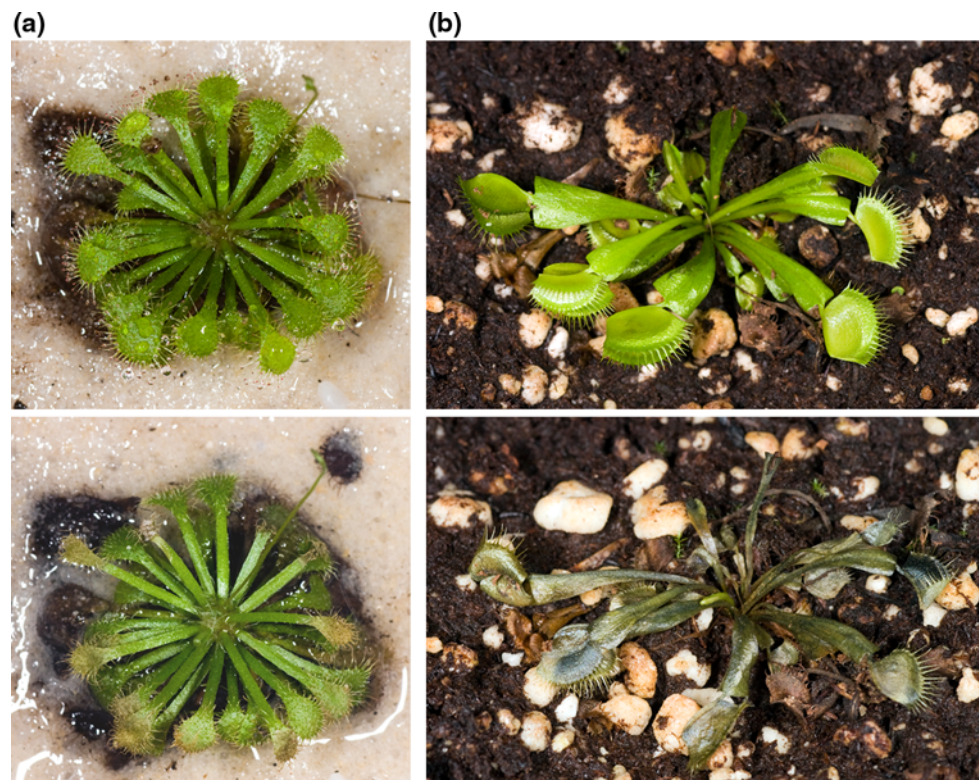
There were significant effects of treatment on survival ( $\chi^2 = 23.64$ ,  $df = 6$ ,  $P = 0.001$ ) and the number of traps/Venus flytrap ( $F = 19.05$ ,  $df = 6, 56$ ,  $P < 0.001$ ). Relative to controls (0% mortality), only commercial grade lambda-cyhalothrin (83.3% mortality;  $P = 0.002$ ) reduced survival (Fig. 1d), but commercial grades of both carbaryl ( $P = 0.003$ ) and lambda-cyhalothrin ( $P < 0.001$ ) significantly reduced the number of traps/plant (Table 1).

## Discussion

Our results demonstrate that both commercial and technical grade insecticides above, within, and below recommended application levels can be harmful to carnivorous plants, justifying agrochemicals as a common threat to these taxa. Insecticides appeared to act on the plants by causing a dieback of mucilage-producing leaves or traps, which in many cases resulted in the death of the plant (Fig. 2). Commercial grades of insecticides were generally more harmful than technical grades, causing significant reductions in sundew and Venus flytrap survival. The more severe effects of commercial grade insecticides could result from their inert ingredients possibly expediting delivery of the insecticide to within the leaf. However, technical grades of insecticide also reduced survival of plants under lab conditions, and frequently caused sub-lethal effects by significantly reducing the expression of traits associated with carnivory.

Pink sundews were generally more susceptible to insecticides than Venus flytraps, although it is important to note that the two species were not directly compared in the same experiment. Pink sundews possess larger numbers of digestive glands on their leaf surfaces than Venus flytraps (Juniper et al. 1989), which could facilitate faster uptake of the insecticides. Considering the sub-lethal effects of insecticides found in both plants, it is possible that there could be long-term consequences of exposure to them. For

**Fig. 2** Comparisons of a pink sundew (*Drosera capillaris*) (a) and a Venus flytrap (*Dionaea muscipula*) (b) before and after treatment with commercial grades of carbaryl and lambda-cyhalothrin, respectively



example, reduced expression of traits associated with carnivory will likely result in fewer prey items being captured, which can subsequently reduce growth and fitness in carnivorous plants (Krafft and Handel 1991). Reduced prey abundance could be an additional indirect effect of insecticides on carnivorous plants, exacerbating their effects on carnivorous traits such as mucilage production. Furthermore, recent evidence has shown that the accumulation of trace metals in invertebrate prey can cause a reduction in the biomass of carnivorous plants (Moody and Green 2010), and similar effects could be caused by insecticides. Given the apparent difference in susceptibility to insecticides between our study species, it would also be prudent to include representatives from a wider-range of carnivorous plant growth forms in future research, such as pitcher plants and fully aquatic species (e.g. many *Utricularia* spp.).

While our results demonstrate that insecticides can be harmful to carnivorous plants, we did not determine the mechanism by which they are damaging the plants. For example, our methods did not allow us to determine exactly which part of the plant is being affected (i.e. leaves or roots), as the insecticides were applied to both the leaves and the substrate. Future research could examine the effects of insecticides when applied exclusively to either the leaves or the substrate in which the plants are growing, thereby isolating their effects on each structure. Additionally, pesticides have been shown to cause damage to the mutualistic mycorrhizae of some plants (Ocampo and Hayman 1980), and while these fungi have not been well-studied in carnivorous plants, there is evidence that they are associated with some species (Fuchs and Haselwandter 2004; Quilliam and Jones 2010). If the application of insecticides to the substrate is found to be more harmful than their application to leaves, then their potential effects on the mycorrhizae associated with carnivorous plants should also be investigated. It could also be beneficial to explore the effects of insecticides on these plants at a molecular level, perhaps by examining the response of enzymes and other proteins to exposure.

Relatively few peer-reviewed studies have examined the phytotoxic effects of the three insecticides used in the present study, making it difficult to generalize our results to other plant taxa. However, there is some evidence that all three insecticides can have negative effects on plants. For example, carbaryl has been shown to reduce the growth of barley (*Hordeum vulgare*) (Murthy and Raghu 1990) and can be phytotoxic to aquatic plants (Peterson et al. 1994), lambda-cyhalothrin has been shown to reduce coleoptile growth in rice (*Oryza sativa*) (Moore and Kroger 2010), and malathion has been shown to be phytotoxic to Sitka spruce (*Picea sitchensis*) and cause a reduction in needle size (Straw et al. 1996). In comparison to these previous

studies, the more frequently observed lethal effects we found with carnivorous plants could therefore be attributed to their digestive glands, and possibly their smaller size than some of the previously studied plants. Consequently, with a range of organophosphate and pyrethroid insecticides used worldwide, broad application of these chemicals could be threatening carnivorous plant species.

## Conclusions

We found that three widely used insecticides can reduce survival and the expression of traits associated with carnivory in carnivorous plants. Given that many carnivorous plant species are found in habitats where insecticides are often directly applied, we recommend longer-term studies on these plants under field conditions to determine the consequences of insecticide exposure at the population-level. Future research should also determine the effects of insecticides on other growth forms of carnivorous plant, such as pitcher plants and aquatic species. Use of these insecticides should be carefully managed in areas containing vulnerable carnivorous plant populations.

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