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Alkaloids and Old Lace: Pollen Toxins Exclude Generalist Pollinators From Death Camas

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ALKALOIDS AND OLD LACE: POLLEN TOXINS EXCLUDE GENERALIST POLLINATORS FROM DEATH CAMAS

by

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Abstract

Many plants produce toxins to which specialist herbivores – typically insects – have evolved counter-adaptations, sometimes resulting in a co-evolutionary arms race. Although many non-social bee species are likewise taxonomic host specialists, the pollination guilds at their floral hosts frequently include diverse floral generalists as well, even on plants that are otherwise chemically defended. In this study, we show that pollen and nectar of foothills death camas (*Toxicoscordion* [=*Zigadenus*] *paniculatum*) contains zygacine, the alkaloid responsible for this plant's notorious mammalian toxicity. Many adults and larvae of the generalist solitary bee, *Osmia lignaria* (Megachilidae), were paralyzed and soon after died when fed biologically relevant doses of zygacine. Such lethality probably explains the absence of this and 50+ other native bee species from this potential host. The sole pollinating bee, *Andrena astragali*, is known to use only death camas pollen to feed itself and its progeny. Thus, pollen and nectar toxins exclude generalist pollinators from foraging at death camas, despite the necessity of pollinators for seed set.

Introduction

Death camas, (*Toxicoscordion* [=*Zigadenus*] *paniculatum*), is a native plant found in the foothills of Cache Valley, Cache Co., Utah. It is distributed throughout the western United States (Garland and Barr 1998). It flowers in spring (April through June) producing one to several racemes bearing pale yellow or white flowers (Figure 1) (Shaw 1989; Shaw 1995). The plant itself resembles an onion plant, and has been eaten mistakenly as such (Havnes 2004). The plant tissue of death camas contains the steroidal alkaloid, zygacine (Figure 2). This alkaloid is known to be toxic to mammals (Garland and Barr 1998).

There are many plants which contain toxins; these toxins are usually withheld from the plant's pollen and nectar in order to encourage insect pollinators. Death camas is unusual in that its pollen and nectar contain the toxin zygacine in an estimated amount of 30 ppm in the pollen, and less in the nectar (Cane and Gardener, unpublished data).

Figure 1. Death camas plant in bloom. Photo by Jim Cane

The only bee known to forage on death camas is *Andrena astragali* (Figure 3). A single species of syrphid fly also visits the flower. Foraging by these two insects seems essential for death camas to set viable seed; in their absence, few fruiting capsules and little seed develop (Jim Cane, unpublished data). Females of *A. astragali* build their nests in the ground. The bee was misnamed in the early 1900s from an observed foraging individual on a plant from the Astragalus family (Tepedino 2003). It is now acknowledged as an oligolege of the Zigadenus family, but its name remains unchanged. It gathers pollen and nectar to make provision masses this is how it should lookfor its progeny; 80% of which has been identified as death camas (Tepedino 2003). Generalist bees, such as *Osmia lignaria* (Figure 4) are not found

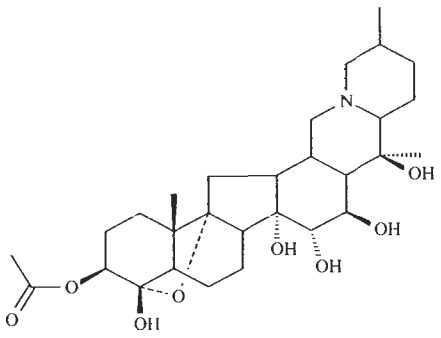


Figure 2. Zygacine, a steroidal alkaloid

Figure 3. *Andrena astragali* foraging on death camas plant. Photo by Jim Cane

Figure 4. The generalist bee, *Osmia lignaria*. Photo by Theresa Pitts-Singer

foraging at death camas. What causes the absence of generalist bees, such as *O. lignaria*, who forage on other plants flowering at the same time? The possibility of zygacine being toxic to generalist bees is explored by this experiment.

An experiment performed by Hitchcock in 1959 indicates that honey bees fed directly on the blossoms of death camas, diluted nectar from, and dissolved pollen from the plant die prematurely (Hitchcock 1959). While his work lays good preliminary research, much information has been learned since then. For example, it is known that the plant is not wind pollinated, as postulated by Hitchcock, but is pollinated by the specialist bee, *A. astragali*. The attempt was to perform a more extensive experiment using *O. lignaria* in the adult and larval stages to solidify Hitchcock's conclusion of death camas having poisonous pollen and nectar.

Methods and Materials

Adult Assay:

For the adult assay, the flower method developed and described by Edith Ladurner was used (Ladurner, Bosch et al. 2003). Materials used were ice cream cups and lids made of wire mesh and Petri dishes, as well as the Styrofoam squares to mount the flower, and vials for the syrup.

Dale Gardner isolated and purified zygacine from death camas foliage in an aqueous solution of 1mg zygacine per 100ml water (1% zygacine). A 50% w/w (weight by weight) fructose: water solution was made and combined with equal amounts of the aqueous zygacine to

Figure 5. Inserting vial into reproductive column of forsythia flower on Styrofoam mount. Photo by Melissa Weber

make a 25% w/w fructose solution with 0.5% zygacine. This 0.5 % solution (50 parts per million, or ppm) was the most concentrated dose. From this, four 10-fold dilutions were made, resulting in 5, 0.5, 0.05, and 0.005 ppm. A control was made of 25% w/w fructose solution with no zygacine.

Male cocoons of *Osmia lignaria*, the blue orchard bee, were obtained. Each assay included six bees: one control, and five for the 10-fold dilution series. For each assay, six flowers (periwinkle or forsythia) were cut and their reproductive columns were replaced with an empty vial (Figure 5). With a micropipette, 10 µl of the appropriate solution were added to each vial (Figure 6). The flowers and Styrofoam mount were then placed into an ice cream cup, along with an emerged bee (generally emerged 1 to 5 days earlier and kept at 4°C). The six bees were observed for one hour, initially in interior lighting. Because feeding was unreliable, the bees were taken to a greenhouse where natural light encouraged foraging activity. Bees were observed for drinking, amount consumed, behavior towards solution, physiological response to dose, and mortality. Mortality was also scored for 24 hours after dosing. The assay was performed 19 times, with 92 out of 114 bees (81%) drinking and giving results.

Figure 6. Placing dose in vial with micro-pipette. Photo by Melissa Weber

Figure 7. Male *Osmia lignaria* drinking dose in vial. Photo by Melissa Weber

Larval Assay:

Nesting blocks were prepared for the larval assay according to the design in Figure 8. A popsicle stick was glued to one end of a block of wood with four grooved holes in it. On top of the block, transparent acetate plastic, fabric padding, and another block of wood were secured with duct tape as a hinge so that the block could be opened. The closed blocks were set outside to allow nesting female *O. lignaria* to fill the holes with provision masses and eggs.

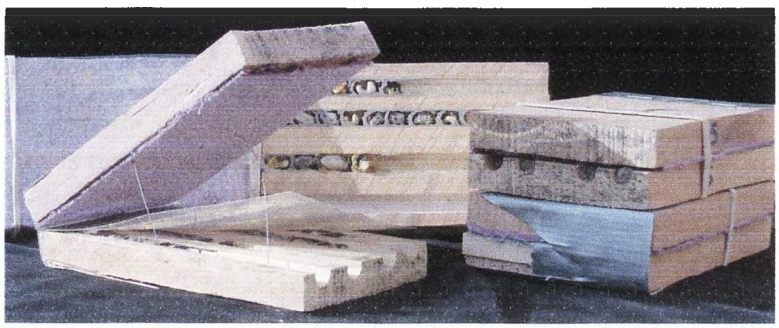


Figure 8. Display illustrating the larvae nesting block and its construction. The foreground shows the grooved board with the plastic cover, fabric, duct tape hinge, and popsicle stick backing the holes. In the background, the grooves are shown filled with provision masses, larvae, and cocoons. Photo by Jim Cane

Once the adult females completed enough nest cells, six blocks were brought in and the provision masses were dosed with 10 µl of solution using the same dilution series as in the adult assay. Several provision masses without eggs were dosed with 10 µl of blue dye to evaluate diffusion into the provision mass. The blocks were then closed and set aside indoors at room temperature. To enlarge the sample size, 7 more blocks were set out to fill up with nest cells, brought in, and dosed.

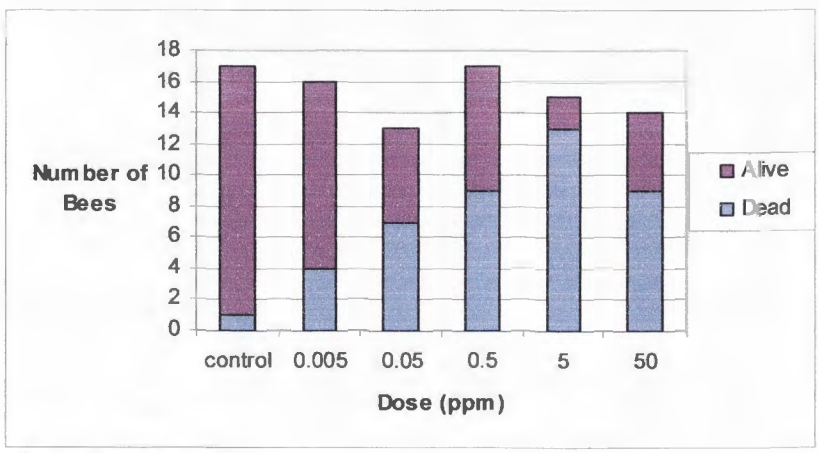
Observations of larva size, feeding behavior, and mortality were taken 12 days after dosing, and again 26 days after dosing for blocks one through six. Observations for blocks seven through thirteen were made 7-9 days after dosing, and again about 25 days after dosing. Both sets of blocks were scored again after completion of cocoon spinning for presence of chalkbrood disease and parasites.

Results

Adult Assay:

The flower and vial method for administering a dose to a bee was fairly effective; however, it had several drawbacks. There was no way of ensuring that a bee would feed on any, or all, of the dose. Eighty-one percent of the bees used for this assay drank at least a portion of their dose. This seemed consistent with the results reported by Ladurner, Bosch et al. (2003). Bees were more likely to drink in natural light, but intense heat caused unreliable feeding.

Often the entire dose was not consumed by the bees. In order to identify a correlation between the dose and the amount consumed, the amount of remaining solution was measured using a capillary tube. The syrup was drawn up into a 34 mm capillary tube that would hold 10 µl. Using a control vial, evaporative loss was taken into account for a more accurate reading. The data for amount consumed was only completed on 29 individual bees, so no analyses were done.



Graph 1. Adult mortality.

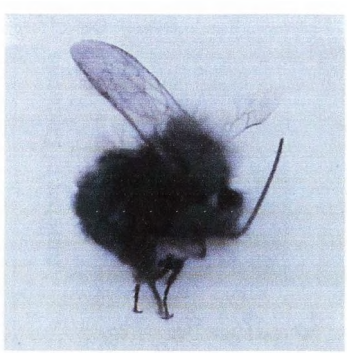


Figure 9. A male *Osmia lignaria* dosed with 50 ppm showing symptoms of zygacine poisoning. Photo by Melissa Weber

Mortality in the control was only 6%, and 25%, 54%, 53%, 87%, and 64% in increasing doses (Graph 1). Bees drinking higher doses often showed symptoms of poisoning such as muscle contractions, lying on their side, writhing, intermittent buzzing, and

paralysis (Figure 9). On two occasions, a bee regurgitated its contents, resulting in a sticky trail of liquid on the bottom of the cup right next to the dead bee's proboscis. These individuals were given doses of 50 ppm and 0.05 ppm. Some bees seemed able to sense, by taste or otherwise, the presence of zygacine. They appeared shocked or startled at the first taste of it, buzzing and flying away suddenly. A bee's apparent ability to sense the toxin may explain the lesser mortality for the 50 ppm dose. If sensing the toxins, by taste or otherwise, the bees refused to drink the full amount, receiving a lower dose than anticipated. This alteration in dose received was not realized until most of the assays were completed, and could not be adjusted for, as remaining male adult bees appeared weak from excessive time spent over-wintering.

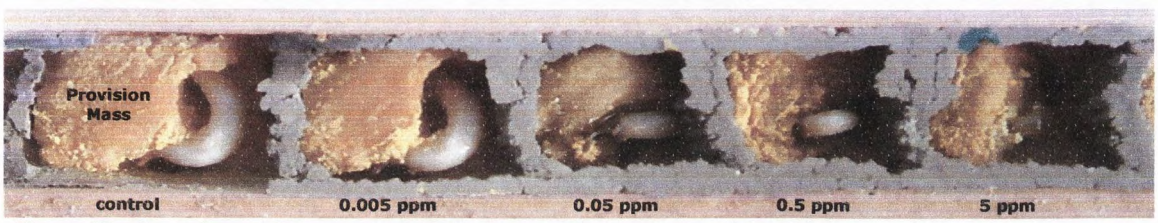
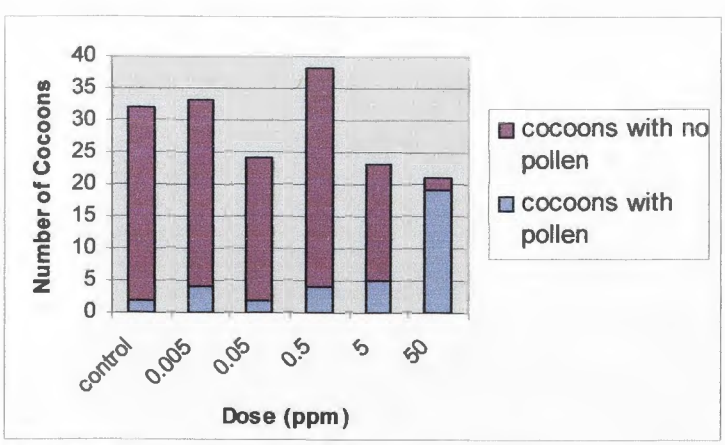


Figure 10. *Osmia lignaria* larvae eating individual provisions in nest cells. A trend can be seen of decreased body size with increased concentration of zygacine. Photo by Melissa Weber

Larval Assay:

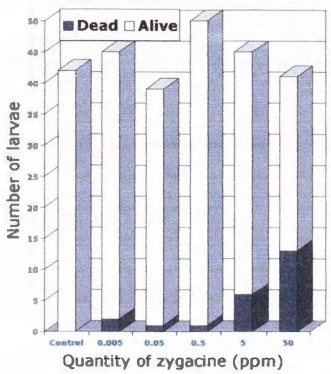
In general, larvae dosed with higher concentrations of zygacine remained small (Figure 10). Small larvae showed distaste while eating by pulling away from their provision mass (n=10). Some larvae that continued to grow spun cocoons before finishing their provision mass (n=36) (Graph 2).



Graph 2. Cocoons spun before finishing provision mass.

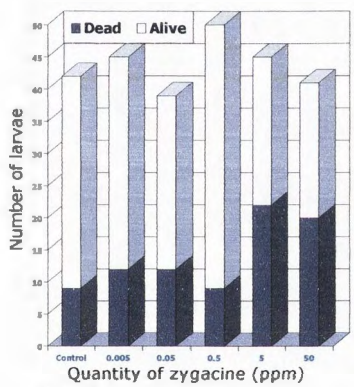
Only two of these were

controls and nineteen were dosed with a concentration of 50 ppm. Early mortality generally increased with dose concentration. Relationships between zygacine dose and larval mortality are shown in Graphs 3 and 4. Mortality was scored twice to take into account a delayed encounter with the dose, as the dye applied was found to diffuse approximately half way through the provision mass.

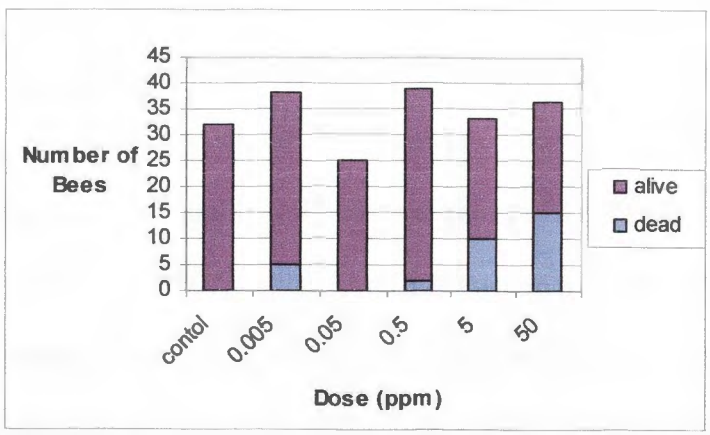


Graph 3. Early larval mortality.

Late mortality for the control and the lowest three doses appeared to be high and about equal. This is explained by the discovery of chalkbrood disease. Chalkbrood is a fungal disease that is passed to a bee larva in its provision mass. An infected bee larva is killed and its carcass is filled with black spores that will be released next spring and contaminate emerging females, who will then infect their progeny. The presence of the disease did not become apparent until blackened carcasses were observable to the human eye. Disregarding deaths due to chalkbrood, late mortality assumed a pattern similar to early mortality (Graph 5). The control and lowest 3 doses are all still close in size, but mortality is much nearer to zero. The last two doses still increase noticeably.



Graph 4. Late larval mortality.



Graph 5. Late larval mortality without chalkbrood.

Discussion

The behavior of both adult and larval bees implies that the bees are sensitive to the taste of zygacine. The adult's sharp reaction suggests that the taste might be unpleasant. In mammals, the toxin appears to irritate mucous membranes (Bloomquist 1999) and stimulate nerve endings in the mouth and throat, causing a tingling sensation as reported by a woman who ate a death camas bulb (Havnes 2004). Perhaps the nerve endings on a bee's proboscis create a similar sensation in response to zygacine. The larvae pulling away from their provision masses might be an indication of feeling unwell after consuming the toxin, rather than a negative reaction to the taste.

During peak emergence time, early to mid April, more males emerged than could be processed. Unused males were kept at 4°C until we performed the assay with them. This delay may have depleted their fat bodies, causing weakened bees and deaths due to starvation instead of zygacine poisoning. By late April, early May, the adults hatching out of cocoons had been over-wintering for an abnormally long period. The males used during this time appeared weak and foraged less reliably. This late emergence may have also increased mortality due to starvation, blurring zygacine's role in mortality. During this time, it was more reliable to observe the presence of symptoms prior to death to ensure the death was due to zygacine.

The next step in this project will be to increase the concentration of zygacine in doses given to adult and larval bees to obtain an LD50 (lethal dose that kills 50% of the population) for zygacine and *O. lignaria*. An LD50 would quantify exactly how toxic zygacine is to this generalist bee. This will require a more concentrated original solution of zygacine, which we have not yet achieved. A higher LD50 for adults than for larvae may reveal that generalist bee adults avoid death camas more for the protection of their progeny than for themselves. However,

the fact that male generalist bees are not seen foraging at death camas indicates that the avoidance is not strictly philanthropic.

The inability of generalist bees to cope with the alkaloid toxin zygacine brings up the question of how the specialist bee, *A. astragali*, physiologically handles the toxin. Veratrum alkaloids, which are similar to steroidal alkaloids, cause attack the nervous system of insects. The reactions include repetitive firings of the axon, an increased negative action potential, and a depolarization of the nerve membrane potential (Bloomquist 1999). A sawfly specialist on a plant containing a veratrum alkaloid is thought to sequester the toxin in one of four ways: degrading the alkaloid, excreting the alkaloid intact, metabolizing and then sequestering, or direct sequestration (Schaffner, Boeve et al. 1994). These could be possible mechanisms occurring in *A. astragali*. This area is currently unresearched.

Mammalian reactions to zygacine include muscle tremors, rapid heart rate, and prostration (Garland and Barr 1998). Birth defects, including cyclopia, cleft palate, and shortening of limb bones, are also seen in mammals as a result of toxic alkaloids (Keeler 1975). Similarly, developmental defects may be seen in bees. Plans for rearing out *O. lignaria* adults from the cocoons of dosed larvae next spring may reveal the presence of such defects.

The mode of evolution which brought *A. astragali* and death camas to their current affiliation is currently unknown. Possibly A. astragali had built up resistance to alkaloid toxins from a different host, and switched over to death camas. If death camas evolved with a pollinator, there would be no need to withhold the toxin from its pollen and nectar to encourage pollinators. Perhaps the plant evolved without a pollinator and was able to self-pollinate or gained cross-pollination through wind travel. To exploit an open niche, individuals of *A. astragali* may have adapted to become resistant to the plant's toxin. Once securing a pollinator,

the plant may have lost its ability to self-pollinate and grew dependent on the bee for seed set. Or, perhaps the specialist syrphid fly played an important role, enabling the plant to be pollinated without a bee until the *A. astragali* species exploited that open niche. These unanswered questions bring up much to ponder on the current status of these two organisms.

Conclusion

Because it is present in the nectar and pollen of the plant, the toxin zygacine is a major factor in keeping generalist bees away from death camas. Adults and larvae of *O. lignaria* suffer symptoms of paralysis and death when administered zygacine in doses ranging from 0.005 to 50 ppm. Symptoms become more common in the adult bees given concentrations of 5 and 50 ppm zygacine. Larval bees administered the same doses exhibit higher mortality and an increase in premature cocoon spinning. Death camas is visited by a syrphid fly and a single bee species, *A. astragali*, who overcome zygacine to specialize on death camas. The mode of action for this tolerance is unknown.

In addition to reaching the overall conclusion of zygacine toxicity, we discovered several behavioral and physiological effects. In the adults, observation revealed distaste, muscle contractions, and paralysis. For the larvae, exposing the typically concealed process of larval development proved educational in addition to revealing the effects of zygacine on developing bees. The larvae, encountering the toxin at different times due to the uneven distribution of the dose, showed symptoms of discomfort and loss of appetite.

Many bees specialize on plants, but usually generalists are welcomed by the plant. When generalists are kept away, it is often due to flower morphology. Rarely are generalists kept away by a toxin contained in the plant's pollen and nectar. *Andrena astragali* and death camas are currently one of our best insect examples of specialization driven by host toxins.

Acknowledgements

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