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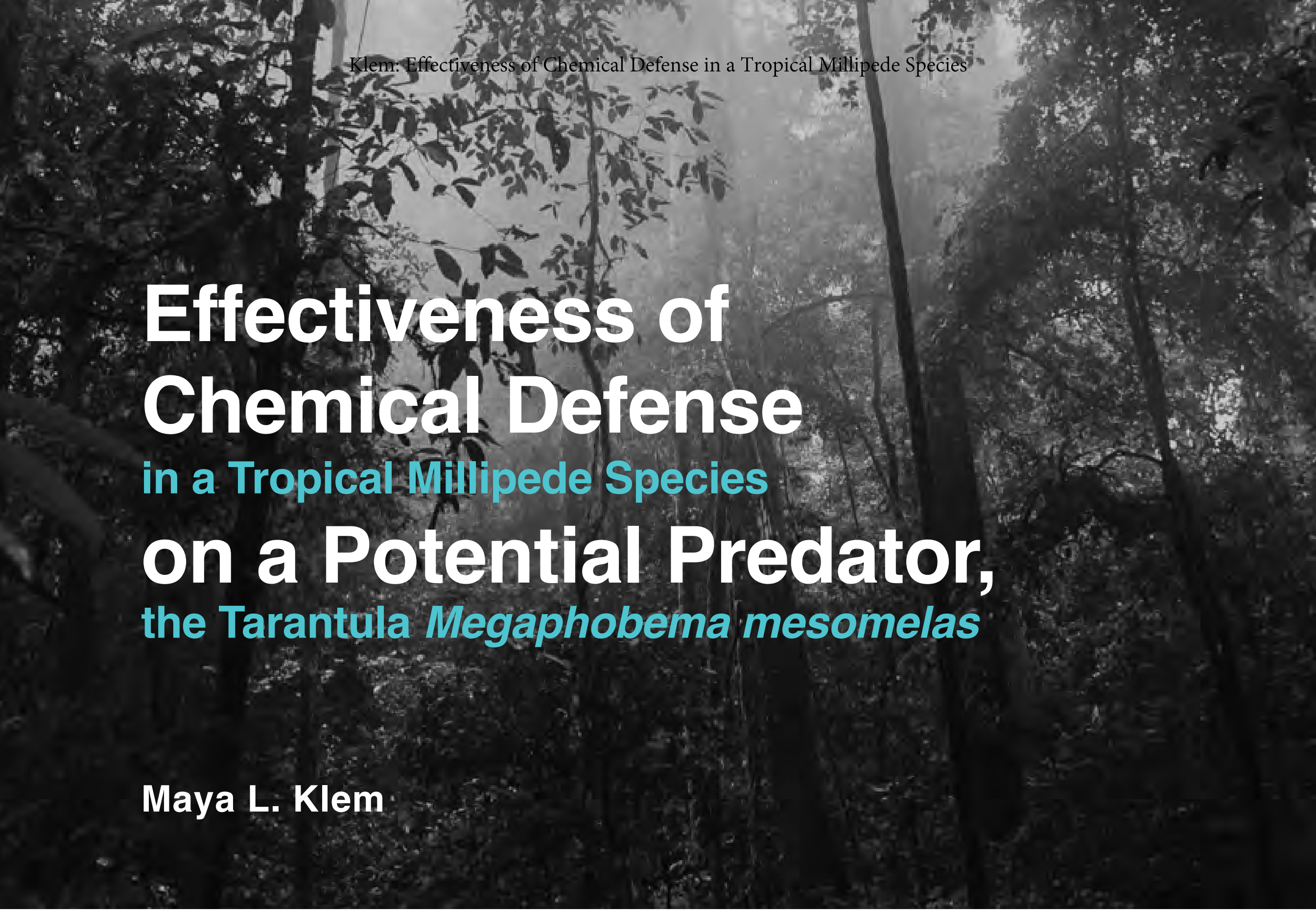
Effectiveness of Chemical Defense in a Tropical Millipede Species on a Potential Predator, the Tarantula Megaphobema mesomelas

Maya L. Klem
Western Washington University, klemm@wwu.edu



Effectiveness of Chemical Defense
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ABSTRACT

Millipedes are known to have a variety of physical and chemical defenses to deter predators. Some species of tropical millipedes have considerably reduced primary defenses when compared to other tropical millipedes, but do retain chemical defense mechanisms. This study uses the tarantula *Megaphobema mesomelas* to test the effectiveness of a secondary defense mechanism, cyanide and benzaldehyde, from a species lacking a robust primary defense. Five tarantulas were found and collected around Monteverde, Costa Rica and brought into the Monteverde Biological Station. They were housed in tanks to allow for controlled feeding trials. Two treatment groups were created: millipedes with cyanide (c-millipedes) and without cyanide (n-millipedes). Each night for 12 nights tarantulas were randomly assigned a treatment and fed a millipede from their corresponding treatment group. It was recorded whether or not the tarantula ate the millipede. It was found that tarantulas had no preference for millipedes with or without cyanide. Tarantulas pounced and began to inject venom into millipedes in less than one second, whereas it took millipedes almost 12 seconds to release cyanide. It appears as though *M. mesomelas* are able to attack faster than the millipedes were able to release cyanide and thus are not exposed to cyanide. For this reason, the millipedes' chemical defense mechanism was not effective in deterring *M. mesomelas*.

Note: a Spanish version of the abstract is available upon request.

INTRODUCTION

Organisms have developed a myriad of defenses to escape predation. In arthropods, there are two categories of defense mechanisms: primary and secondary. Primary mechanisms include passive defenses such as speed, tough exoskeletons, shelters, and camouflage; secondary mechanisms consist of chemical defense (Borror et al. 1989).

Diplopoda, commonly known as millipedes, have evolved a variety of defense mechanisms. There are over 12,000 described species of millipedes in the world (Golovatch & Kime 2009; Sierwald & Bond 2007). As detritivores, these slow-moving creatures live on the forest floors (Brusca & Brusca 1990). Though millipedes lack venom, many species have a variety of primary defenses including a thick exoskeleton and the ability to roll into a tight ball (Heisler 1983). Many also have secondary defenses such as the ability to secrete toxic and volatile compounds. Some millipedes contain a non-muscular repugnatorial gland that oozes or secretes irritating or toxic compounds. These compounds are made in vivo, released all at once, and take between two weeks and four months to regenerate; they deter predation in varying ways ranging from irritating the eyes of mammalians predators to burning arthropods' exoskeletons (Shear 2015).

Most of these 12,000 species are endemic to the tropics (Golovatch & Kime 2009). In Costa Rica, one of the most well studied species of millipede is *Nyssodesmus python*; they possess a very thick, calcified exoskeleton and the ability to roll up into a tight ball. In addition to these primary defenses, *N. python* have the ability to spew hydrogen cyanide and benzaldehyde up to 30 cm to ward off predators. When these defenses are combined, the result is almost no predation of this species. The only common causes of mortality for adult *N. python* are parasites, desiccation, or injury in the delicate post-molting stage (Heisler 1983; Sierwald & Bond 2007). However, other millipede species' common predators are ants, beetles, predatory arthropods, spiders, slugs, and some visually hunting vertebrates (Shear 2015; Sierwald & Bond 2007). One species of millipede found in Costa Rica lacks *N. python*'s primary defense mechanisms—a thick exoskeleton and the ability to curl into a ball. Despite the appearance of reduced primary mechanisms, millipedes seem to expel a similar secondary compound. The millipedes in this study were experimentally confirmed to contain cyanide, which is produced in a 1:1 molar ratio with benzaldehyde in their repugnatorial glands (Shear 2015). With reduced primary defenses, this smaller millipede species may be more reliant on secondary defenses.

Tarantulas are opportunistic sit-and-wait predators that could potentially prey on millipedes. Additionally, tarantulas have regions on their pedipalps (Fig. 1), a pair of secondary appendages used in feeding, and in some cases front legs that are capable of chemical sensation or taste (Perez-Miles 2005). They rely on this taste mechanism for hunting, which could make their prey's chemical defense effective. Thus, tarantulas are a good predator to test the effectiveness of a millipede's secondary defense mechanism.

To explore this, a common tarantula species found in parts of Costa Rica, *M. mesomelas*, were captured and housed in a controlled environment to study their reactions to a millipede's secondary defense strategy. Five tarantulas were repeatedly fed millipedes (species unknown) with and without their chemical defense intact for a total of 12 days and their feeding choices were recorded.

PREDATION:

A relationship between two organisms in which one of them acts as predator that captures and feeds on the other organism that serves as the prey

ARTHROPOD:

Invertebrate animal with a hard, external skeleton and jointed appendages

DETRITIVORE:

Organism that consumes decaying materials, often on forest floors

REPUGNATORIAL GLAND:

Gland from which many insects secrete toxic chemicals

ENDEMIC:

limited to one area of the world

DESSICATION:

the act of drying out

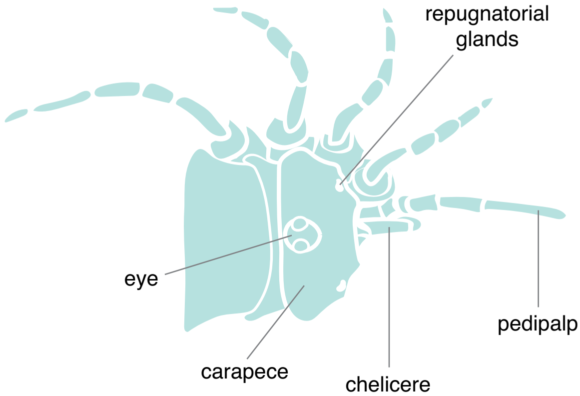

FIGURE 1

MATERIALS AND METHODS

Study Site and Organisms

This study took place in July 2016 between 1300–1500 meters in the premontane wet forest of Monteverde, Costa Rica. Approximately 120 millipedes were collected both in the Monteverde Cloud Forest and around the town of Monteverde. After millipedes were collected and brought into the lab, they were placed in an aquarium with dirt, leaf litter, and rotting logs to allow them to eat.

Five Costa Rican Red-Legged Tarantulas, *Megaphobema mesomelas*, were collected along dirt road embankments around Monteverde, Costa Rica. The tarantulas are from the same life zone as the millipedes and have been observed sharing the same microhabitat. These tarantulas were initially found after sunset by locating holes on steep, dirt embankments along roads. After dark, tarantulas can be seen easily as they are at the edge of their holes waiting for prey. A small stick was used to simulate an insect by lightly tickling one of the tarantula's legs. When the tarantulas felt the stick, they lunged forward. A spoon was then slid behind to simultaneously block their hole and lure them out into a plastic container for transport back to the controlled environment of the lab. The tarantulas were placed in separate aquariums approximately three times the size of their leg span. The aquariums were filled with dirt and each contained a small amount of PVC piping to simulate a hole for the tarantulas (Marshall 2001). Additionally, each tarantula was given a name (Kurt, Zachary, Katti, Demi, and Darryl) for the duration of the study.

Quantifying Millipede Defense

To quantify the primary defense mechanisms of this unidentified millipede species, the mass, length, and width of 25 millipedes were recorded. In addition, millipedes were manipulated to see if they engaged in ball rolling, a common primary defense mechanism in millipedes. To assess secondary defenses, five millipedes were tested for cyanide using sodium picrate test strips. Strips were prepared by creating a solution with 2.5 g sodium carbonate, 1 g picric acid (0.5% w/v, moist), and 100 mL water (Yeh 2014). Filter paper strips (8 cm x 1.5 cm) were saturated in the sodium picrate solution and excess liquid was evaporated. A millipede was then placed in a plastic bag with a sodium picrate test strip and manipulated until it released the chemical; a positive result was indicated by the test strip changing from bright yellow to orange/ red, corresponding with the presence of cyanide. The test strip was dunked in 5.0 mL of deionized water 30 times and then the diluted solution was transferred to a plastic cuvette. A blank solution (without cyanide) was created by dunking an unused sodium picrate test strip in 5.0 mL of deionized water 30 times. The cuvette was measured in a spectrophotometer against a blank sample at a wavelength of 540 nm to determine the percent transmittance of the sample (Lian & Hamir 1981). The concentrations of cyanide and benzaldehyde were then calculated based on the 1:1 molar ratio of cyanide and benzaldehyde production in millipedes' repugnatorial glands (Shear 2015).

Feeding Trials

Diplopoda have a gland that secretes many compounds, including cyanide. However, their cyanide is released all at once, after which it takes at least two weeks for the gland to produce more cyanide (Shear 2015). Knowing this, the millipedes were divided into two categories: those with and without cyanide available. The group without cyanide was obtained by inducing cyanide expulsion with the same procedure used to test for cyanide; the amount of time it took for the millipede to release cyanide was recorded. In the second treatment group the millipedes retained their cyanide. Each night at 18:00 the tarantulas were fed a millipede from one of the two treatments. Both the tarantulas' reactions and whether or not they ate the millipede were recorded. During the 12-day study, each individual tarantula was offered millipedes both with and without cyanide multiple times. If the tarantula did not eat the millipede, they were offered a second meal of a cockroach in order to determine if the tarantula's rejection was due to a lack of hunger or an aversion to the specific millipede; these reactions were recorded. On July 29, 2016, the tarantulas were released back to their original holes.

RESULTS

Quantifying Millipede Defense

The millipede species used in this study is smaller in size and lacks the thick exoskeleton of *N. python* (Fig. 2b). A sample of 25 millipedes were measured to determine their masses, lengths, and widths. Millipede weight ranged from 0.311 g to 0.881 g, length ranged from 32.41 mm to 49.03 mm, and width ranged from 3.90 mm to 7.57 mm (Table 1). The smaller millipede species also did not exhibit the curling defense mechanism that *N. python* show (Fig. 2c). The sodium picrate test for cyanide had a positive result for all five of the millipedes tested, experimentally confirming that the species of millipede used in the study have a secondary defense mechanism (Fig. 2a). The solution had an average transmittance of 39.8% ± 6.02% at 540 nm in the spectrophotometer. Absorbance was determined (absorbance = 2 - (% transmittance)) and used to quantify cyanide per millipede (y = -1.0110 + 371.4679x + 167.4901x2), where y equals the amount of cyanide in µg and x equals the absorbance. The average amount of cyanide per millipede was found to be 187.25 µg ± 31.94 µg (Lian & Hamir 1981).

LIFE ZONE:

a location characterized by the geographical location in addition to the organisms living there (Holdridge 1967)

SPECTROPHOMETER:

a scientific instrument that measures the percent of light that a liquid allows through

WILCOXON PAIRED-SAMPLE TEST:

a statistical test used to determine if the difference in two sets of data are due to an experimental manipulation, or due to chance

FIGURE 2
Comparison of primary and secondary defense mechanisms between *N. python* and the species of millipede used in this study. (a) Sodium picrate cyanide test result of a control strip (bright yellow) and a test strip after being exposed to cyanide released from a millipede (brick orange). (b) Size difference and presence of exoskeleton between the millipede used (left) and *N. python* (right). (c) Example of ball curling as a primary defense in *N. python*.

Feeding Trials
A total of 58 millipedes were offered to the tarantulas: 21 out of the 29 c-millipedes (72%) and 20 out of the 29 n-millipedes (69%) were consumed (Fig. 3). When looking at individual tarantulas, Kurt consumed three of the five c-millipedes and all seven of the n-millipedes offered (Fig. 4). Zachary consumed six of the seven c-millipedes and three of the n-millipedes offered. (Fig. 4). Katti consumed three of the seven c-millipedes and two of the five n-millipedes offered. (Fig. 4). Demi consumed all five c-millipedes and all five n-millipedes offered. (Fig. 4). Darryl consumed two of the four c-millipedes and three of the six n-millipedes offered. (Fig. 4). Demi and Darryl were fed two fewer times because they were captured two days after the other three individuals.

TABLE 1
Average millipede size measurements

Mass	Length	Width
0.5597 ± 0.0400 g	38.5809 ± 1.1448 mm	6.0819 ± 0.2069 mm

A Wilcoxon paired-sample test was conducted to compare individual tarantula feeding preferences for the five tarantulas fed daily during the study. The tarantulas showed no aversion against millipedes with cyanide (N=5, t=3 z=0 p=1) (Fig. 4).

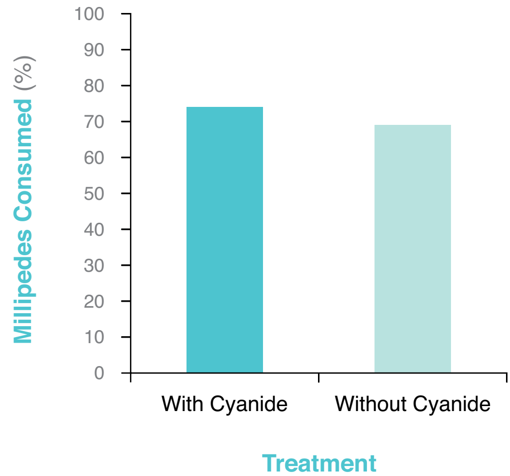

FIGURE 3
Percent of 118 millipedes consumed by five *M. mesomelas* based on cyanide presence.

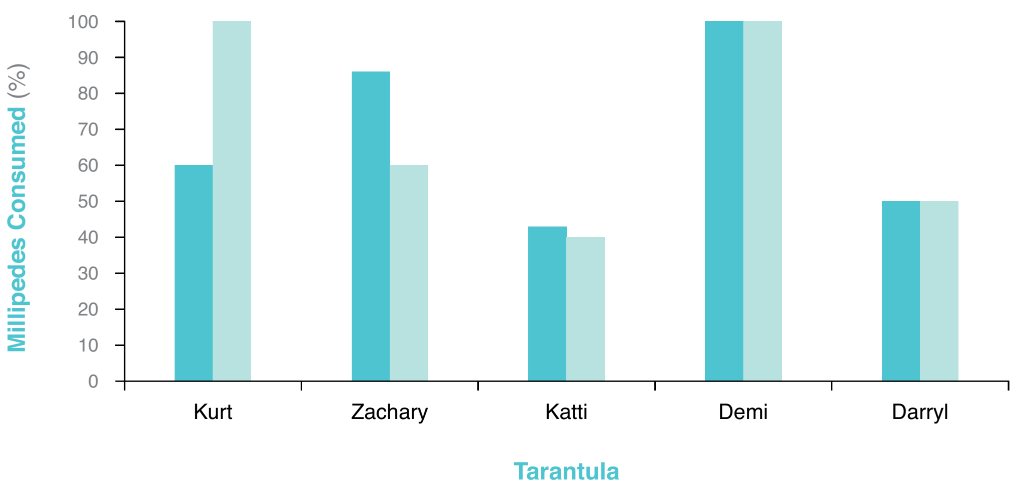

FIGURE 4
Percent of millipedes, with and without cyanide, eaten by each individual *M. mesomelas*. Twelve millipedes were fed to Kurt, Zachary and Katti over 12 days and 10 millipedes were fed to Demi and Darryl over 10 days.

Tarantulas rejected the millipedes 19 out of the 58 feeding trials. Furthermore, 50% of the time tarantulas ate the second meal after rejecting the initial c-millipede and 50% of the time did not eat the second meal after rejecting the initial c-millipede (Fig. 5). By contrast, 11% of the time tarantulas ate the second meal after rejecting the initial n-millipede and 89% of the time did not eat the second meal after rejecting the initial n-millipede (Fig. 5).

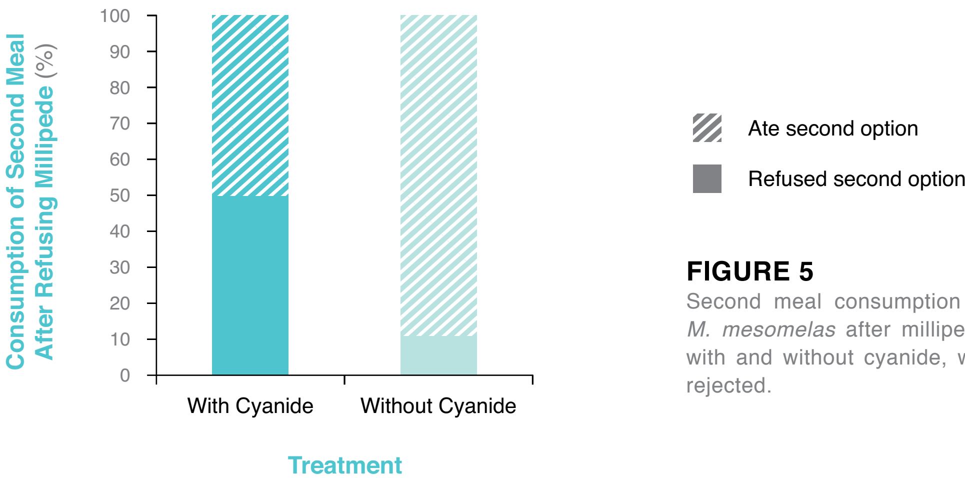

FIGURE 5
Second meal consumption by *M. mesomelas* after millipede, with and without cyanide, was rejected.

If a tarantula pounced on a millipede, it occurred in less than one second (Fig. 6). On average it took millipedes between 1.4 and 79.2 seconds to release cyanide (11.67 ± 2.60 sec) (Fig. 6). If the tarantula did not pounce and attack upon the initial touch of the millipede, it did not consume the millipede. When this happened, the tarantula either had no reaction or backed away from the millipede.

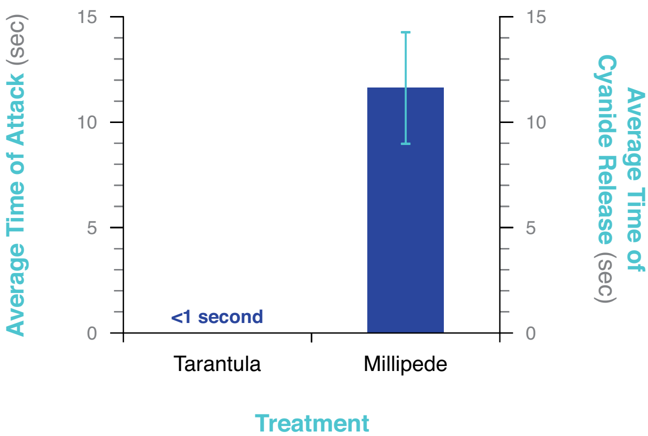

FIGURE 6
Time of attack by *M. mesomelas* compared to the average time millipedes took to release cyanide. The error bar represents one standard error.

Additional Observations

On July 25, 2016 the millipede fed to Zachary released cyanide, which appeared to injure him. He did not move for many hours, even when prodded. The following day, when the millipede was presented to him he pounced and then immediately retracted, which was abnormal behavior for him. The same millipede was presented one more time to him on July 26, 2016 and he once again pounced and retracted. When presented with a second food option, a cockroach, he immediately pounced and consumed it. It should also be noted that Katti is a brooding female who had her egg sac in the tank with her for the study. Due to time constraints, the tarantulas in this study were fed more frequently than they would eat in the wild. All five tarantulas maintained a strong appetite despite their increased food intake. As mentioned in the methods, steps were taken to determine if a tarantula was not eating the millipede or was simply not hungry.

DISCUSSION

The results of this study show that the reduced primary defense mechanisms, such as a thin exoskeleton or the inability to roll into a ball, make millipedes vulnerable to predation. In addition, though they possess a secondary defense, the millipedes are unable to expel cyanide quickly enough to deter sit-and-wait predators such as *M. mesomelas* tarantulas. It appears as though these tarantulas are able to pounce, attack, and inject their venom into the millipedes before they are able to react. Tarantulas pounce quickly to deliver a lethal dose of venom to their prey, which are then liquefied, sucked, and digested (Kosiba et al. 2014). Spiders, including *M. mesomelas*, do have the ability to sense chemicals using patches on their pedipalps (Perez-Miles 2005). This suggests that tarantulas can detect chemicals such as cyanide if they are present. However, the tarantulas ate 72% of millipedes with cyanide and 69% of millipedes without cyanide. Additionally, the tarantulas often did not eat the millipedes from either treatment simply because they were not hungry. When they ate, *M. mesomelas* pounced and began injecting their venom in less than one second. However, the millipedes took on average 11.67 seconds to release cyanide. Logistically, this explains why the tarantulas seemed to be relatively unphased; millipedes did not have enough time to expel cyanide.

The millipedes used in this study were found to contain approximately 187.25 µg of cyanide. This is equivalent to the lethal dose for a 25 g mouse and nearly six times the lethal dose for a 300 g pigeon (Shear 2015). Most likely, the millipedes used in this study also produce benzaldehyde. The gland that produces cyanide has two chambers; one chamber contains mandelonitrile, which is catalyzed to produce benzaldehyde and hydrogen cyanide in the second chamber (Shear 2015). These two chemicals combined are known to be an almost perfect pair in defending millipedes. Hydrogen cyanide does not appear to repel many arthropods, such as ants, whereas benzaldehyde does. Cyanide appears to be an effective deterrent of vertebrates but not of arthropods (Shear 2015). However, these chemicals are essentially useless if there is not enough time between threat arrival and paralysis/death to release them, such as with *M. mesomelas*.

Millipede secretions are known to cause eye irritation or blindness in vertebrates and burn arthropod exoskeletons (Shear 2015). However, tarantulas have a chitin layer covering their eyes that could potentially protect them from these chemical irritations (Pérez-Miles 2005). It is possible that tarantulas are less likely to be affected by these toxic chemicals due to their pedipalps and protective eyes. Noting the instance of cyanide exposure for Zachary, their defenses appear to be insufficient. He retracted from the millipede upon exposure and did not move for multiple hours even when poked, and he appeared to be afraid of the next millipede offered to him. This seems to indicate that if millipedes did have enough time to release chemicals it would likely be effective against *M. mesomelas*.

A similar study conducted on *M. mesomelas* found that they did not have an apparent aversion to a toxic stick bug species. These bugs also spray a toxin, limonene, which can be fatal to insects in as little as 15 minutes. Koranda hypothesized that this was due to the overall larger size of *M. mesomelas* (Koranda 2013). The findings of this study suggest that it is not only the larger size of the tarantula that allows them to eat prey containing poisonous chemicals but also their speed. The Koranda study should be repeated while taking time of attack into account.

Five nights were spent at the beginning of this study finding and capturing tarantulas in Monteverde, Costa Rica. During this period only five *M. mesomelas* were discovered. This indicates that there does not appear to be many *M. mesomelas* in the area. Although these millipedes and tarantulas share the same habitat, it is logical to believe that these millipedes do not come into contact with *M. mesomelas* often. If this is true, there have most likely not been significant evolutionary pressures for these millipedes to evolve a mechanism to evade tarantulas.

BROODING:

when a female animal is caring for her egg sac

CHITIN:

a fibrous compound used for protection and support in many arthropods

CONCLUSIONS

The reduced primary defense mechanisms in this species of tropical millipede, combined with a delayed chemical secondary defense mechanism, does not appear to be effective in deterring *M. mesomelas* predation. However, it is hypothesized that this is due to the incredibly fast attack time of *M. mesomelas* rather than the chemicals not being effective. It is likely that if the millipedes were presented to a slower predator, their chemical defense would be effective, as it is with other animals of similar or larger sizes than *M. mesomelas* (Shear 2015).

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Department of Biology
Western Washington University
Professor: Dr. Adam Stein
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REFERENCES

Borror, D. J., C.A. Triplehorh, & N.F. Johnson. (1989). An introduction to the study of insects, ed. *Saunders College Publishing:* 78–79.

Brusca, R. C., & G.J., Brusca. (1990). Invertebrates. Sunderland, MA: Sinauer Associates: 544–591.

Golovatch, S. I., & R.D., Kime. (2009). Millipede (Diplopoda) distributions: a review. Soil Organisms, 81: 565–597.

Heisler, I. L. (1983). Nyssodesmus python (Milipies, large forest-floor millipede). *Costa Rican Natural History,* D.H. Janzen, ed. The University of Chicago Press: 747–749.

Holdridge, L. R. (1967). Life zone ecology. Life zone ecology., (rev. ed.)

Koranda, K. W. (2013). Behavioral and feeding responses of the tropical tarantula *Megaphobema mesomelas* (Theraphosidae) to the toxic stick insect anthericonia anketeschkei (Pesudophasmatidae). *CIEE: Tropical Ecology and Conservation*. Summer 2013: 94–100.

Kosiba, S., P. Allen, & G. Barrantes. (2014). Feeding effectiveness of *Megaphobema mesomelas* (Araneae, Theraphosidae) on two prey types. *Arachnology.* 15:228–230.

Lian, T. S., & N.A. Hamir. (1981). Spectrophotometric quantification of Guignard's sodium picrate test. *MARDI Research Bulletin*. 1:35–41.

Marshall, S. D. (2001). Tarantulas and other arachnids: Everything about purchase, care, nutrition, behavior, and housing. Hauppauge, NY: *Barron's Educational Series.*

Pérez-Miles, F., F.G., Costa, C., Toscano-Gadea, & A., Migone. (2005). Ecology and behaviour of the 'road tarantulas' Eupalaestrus weijenberghi and *Acanthoscurria suina* (Araneae, Theraphosidae) from Uruguay. *Journal of Natural History.* 39: 483–498.

Shear, W. A. (2015). The chemical defenses of millipedes (Diplopoda): biochemistry, physiology and ecology. *Biochemical Systematics and Ecology.* 61:78–117.

Sierwald, P., & J.E., Bond. (2007). Current status of the myriapod class Diplopoda (millipedes): taxonomic diversity and phylogeny. *Annual Review of entomology.* 52:401–420.

Yeh, A. (2014). Within plant cyanide allocation in Passiflora spp. *CIEE: Tropical Ecology and Conservation.* Summer 2014: 86–94.