Evaluation of mechanical defense provided by pericarps of three different *Crotalaria* species to their seeds against a specialist herbivore, *Utetheisa ornatrix*: a case for a possible host-herbivore evolutionary arms race

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Abstract: Past studies that explored the evolutionary arms race between toxic *Crotalaria* plants and their herbivores have mostly focused on chemical co-evolution. In this highly speciose genus of plants, we hypothesize that other defenses, such as mechanical protection of the seeds, which are exploited by specialist herbivores for their nutrients and alkaloids, have also been evolving, together with the herbivores' ability to overcome them. To test this hypothesis, we assessed the cost of penetration imposed on a specialist herbivore, the larvae of the Ornate Bella Moth *Utetheisa ornatrix*, by the pericarps of three *Crotalaria* species. We tested the Florida native *C. pumila*, which has supposedly been in co-existence with *U. ornatrix* in the New World for thousands of years, the introduced species *C. spectabilis* that is native to Asia, and another introduced species, *C. pallida*, which is native to Africa, where multiple tiger moth species, including ones ancestral to the genus *Utetheisa* such as the genus *Amphicallia*, attack *Crotalaria*. Our evaluation was based on the ability of larvae to penetrate pods, their mortality, the rate of development of 4th through ultimate instar larvae when reared on open vs. closed pods as diet, and the wing span of resultant adult moths as a measurement of fitness. The cost associated with larval penetration of pericarps was greatest when feeding on *C. pallida*, followed by *C. pumila*, with the cost of penetration of *C. spectabilis* barely detectable in our study. We found that there are important structural and mechanical differences in the cellulose fibers forming the inner layer of the pericarps, which could help explain these differences in cost of penetration. Similar research on other *Crotalaria* species and associated tiger moths is required to test the evolutionary arms race hypothesis.

Key words: arthropod-plant interaction, herbivore-plant interaction, insect-plant co-evolution, New Associations Hypothesis, plant mechanical defense

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INTRODUCTION

Plants use a variety of defense mechanisms to ward off herbivores. These defenses are divided into two broad categories: chemical and mechanical. Chemical defenses include using toxic and indigestible substances (Wink & Mohamed, 2003), and chemicals that simulate insect hormones and affect insect development (Fowler *et al.,* 2001), act as glue that impairs feeding (Harvey *et al*., 2011), or signal to parasitoids (DeMoraes *et al.*, 1998) and predators (Guimarães *et al.*, 2006). There are also various mechanical defenses, such as physical barriers that keep predators from consuming the plant tissues, such as hairs, spikes, wax layers, and hard walls (Becerra *et al*., 2001). However, insects constantly evolve ways to overcome this resistance and these arms races often lead to reciprocal evolutionary change (e.g., Ehrlich & Raven, 1964). Recently, Edger *et al.* (2015) discussed co-evolutionary, genetic and molecular mechanisms of this arms race and concluded that the evolution of both plant defenses and resulting adaptations in herbivores are associated with changes in diversification rates. Legumes in the genus *Crotalaria* (rattlebox plants) (Fabaceae) and the tiger moths that specialize in feeding on them is one of the many putative examples of plant-herbivore co-evolution.

There are over 700 species in the genus *Crotalaria*, which are found primarily in tropical and subtropical regions of the world. Some *Crotalaria* species are native to the New World, while some were introduced by humans in part because of their ability to fixate nitrogen and improve soil quality (Austin, 2004). These plants contain pyrrolizidine alkaloids (PAs), which are toxic to many animals, including horses (e.g., LSUAgCenter, 2012), sheep (Maia *et al.*, 2014) and humans (e.g. Williams & Molyneux, 1987; Diaz, 2014). Most insects do not feed on *Crotalaria* because of the harmful PAs. There are several exceptions, however. The most notable among them in the New World is *Utetheisa ornatrix* (Ornate Bella Moth) (Erebiidae: Arctiinae). By consuming *Crotalaria* the larvae of this moth ingest alkaloids, which render them poisonous to many predators (e.g., Eisner & Eisner, 1991; Martins *et al.,* 2015) and provide them with precursors for sex pheromones (e.g., Conner *et al*., 1981; Conner, 2008). Larvae actively seek the individual plants and tissues of the plants that contain the highest concentration of PAs (Hoina *et al.,* 2013), and Walsh and Iyengar (2015) showed that competition for the seeds, the richest source of alkaloids in a plant, favors larger larvae, and is affected by the relatedness of the larvae, with siblings more likely to maintain control of the resource over non-sibling competitors. Cogni *et al.* (2012) found that there is very little cost to *U. ornatrix* in the sequestration of PAs, and hence larvae past third or fourth instar are attracted to seeds where PA concentration is highest, regardless of the *Crotalaria* species.

Paradigm

Despite not being negatively affected by the toxins, larvae must nevertheless find a way to work through the pericarp to reach the seeds, and it has been proposed that the plants have evolved mechanical defenses in their pericarp to prevent larvae from reaching the seeds (Ferro *et al.,* 2006; Sourakov, 2015). A former study demonstrated the high cost in the form of larval mortality of penetration of *C. pallida* pericarps by young *U. ornatrix* larvae. It has also been proposed that not only mechanical defenses, but also the arrangement of seeds on a plant, such as the number of seeds per pod and whether pods are single or clustered, might affect how efficiently larvae obtain nutrients and PAs from different *Crotalaria* species (Sourakov, 2015). In the present study, we compare these defenses in three *Crotalaria* species in Florida: one native and two exotics.

Pericarps in two of the more common Florida native *Crotalaria*, *C. pumila* and *C. rotundifolia*, are quite hard, resembling peanut shells in consistency, and enclose few seeds that are more watery and less nutritious (including lower PAs) than those of the exotics (Sourakov, 2015). For example, at the same stage of development, *C. pumila* seeds are 89% water vs. 79% in African *C. pallida* and 77% in Asian *C. spectabilis*; PAs vary in different populations of *C. pumila* from 0.04% to 0.6%, while in *C. spectabilis* they can be as high as 3.81% (Sourakov, 2015; Williams & Molyneux, 1987; Burkill, 1995; Hartmann *et al.*, 2004). A larva feeding on the native *C. pumila* and *C. rotundifolia* would presumably have to expend a significant amount of energy breaking into these pods in order to consume the nutrients and PAs required to complete their development (see for example Fig. 3 and a supplementary video at https://youtu.be/uA0BuWzqxOc).

In nature, the task will be additionally complicated by the fact that these pods are scattered about the plant and each plant produces only a few of them, so seeking the pods out would also require energy and be a risky endeavor, as it would involve crawling on the ground. In contrast, seeds of the Asian *C. spectabilis* are surrounded by softer pericarps akin to green peas. This species' high alkaloid content may be a response to different types of herbivores, as the plant is clearly extremely toxic to vertebrates (e.g., Yan & Huxtable, 1995). Finally, *C. pallida* and *C. lanceolata*, which are similar species introduced to Florida from Africa, have relatively low PA concentration in seeds (ca. 0.2% according to Williams and Molyneux (1987)), and produce large pods that grow in clusters, where a single cluster can satisfy the nutritional requirements of a maturing larva. Their pericarps, soft when the seeds are still small, later, when the seeds are the most beneficial to larvae, become very hard, rubbery and difficult to crack open (pers. obs.).

According to Le Roux *et al.* (2013), *Crotalaria pallida* forms part of an early diverging lineage in a section that is mostly restricted to Africa. Both *C. pumila* and *C. spectabilis* are later diverging and found in a section that is mostly extra-African. There are a number of *Crotalaria*-feeding arctiines in Africa, including, for example, the large and widespread genus *Amphicallia*, which is ancestral to *Utetheisa* (*sensu lato*) according to DaCosta and Weller (2005). Therefore, *C. pallida* plants have likely been under selection pressure from such larvae for a significant period of time and can be expected to have evolved better defenses against them. The geographic association of *C. spectabilis* with *Crotalaria*-feeding *Utetheisa* in Asia, where some members of *Utetheisa* feed on non-*Crotalaria* hosts, is likely to be the most recent among the three tested species, as might be implied from phylogenetic studies (DaCosta, 2010; Le Roux *et al*., 2013).

The present study therefore compares the ability of pericarps of three different species of *Crotalaria* that grow naturally in Florida to protect their seeds from *U. ornatrix* larvae. We tested the efficiency of *U. ornatrix* larvae from the fourth instar onwards in penetrating pericarps of the native *C. pumila* and the exotic *C. spectabilis* and *C. pallida*, and discuss reasons for the observed differences.

MATERIALS AND METHODS

Host plants were available on the University of Florida campus in Gainesville. Experiments were conducted in the fall of 2014, during which time seeds were in an appropriate stage of development. Younger larvae normally feed exclusively on leaves, hence in our experiments we used larvae of early fourth instar and older.

Three separate experiments were conducted:
- Experiment 1 tested native *Crotalaria pumila* and exotic *C. spectabilis* simultaneously
- Experiments 2 and 3 tested exotic *C. pallida*

For the purpose of reducing genetic variability within each of our trials, we used a split-brood design in experiments 1 and 2 (experiment 3 was conducted using a batch of field-collected larvae). For Experiments 1 and 2, the two parental moths (fem#1 and fem#2) were netted behind the Florida Museum of Natural History, Gainesville, Florida (29°38.0' N, 82°22.2' W) on 22 Oct 2014. That population is associated mostly with *C. lanceolata*, yet *C. pumila*, *C. spectabilis* and *C. pallida* are also available to that population, although in smaller numbers. The moths were fed a sugar and water solution and were kept in individual 2 oz. clear plastic cups, in which they laid their eggs. The larvae were raised inside such cups on *C. lanceolata* leaves that were changed three times a week.

At the 21st day of their development, when offspring larvae of fem#1 reached late 3rd-early 4th instar, 75 of them were divided into 5 groups - 15 larvae per group. Three groups were provided with *C. pumila* as follows: group (a): closed pods; group (b): pods cracked open; and group (c): leaves. Two other groups were provided with *C. spectabilis*: group (d): closed pods; and group (e): pods cracked open. As the larvae varied slightly in size, we took special care to divide larvae equally according to size, so that the groups were as close to identical as possible at the onset of the experiment.

All groups were raised subsequently with only one larva per 2 oz. cup that received individual voucher numbers. Plastic cups were cleaned daily and food was made continuously available in abundance, so that its shortage never occurred.

For each individual larva, dates were recorded of larval death, pre-pupation, pupation, and emergence. Larvae were weighed using a Mettler Toledo AL104 analytical balance on 25 Nov 2014, 11 days into the experiment, to provide an additional reference point for assessing their development. Once the adult

moths emerged from pupae, they were frozen and then spread. Electronic calipers were used to measure forewing (FW) length from wing base to tip of dry voucher specimens, which were labeled and deposited in the collection of the McGuire Center for Lepidoptera Research, Florida Museum of Natural History, Gainesville (MGCL database numbers 166318-166399). Two-way ANOVAs and a Paired T-test were conducted to compare the duration of larval development, pupal development, and FW length, with males and females treated as separate subgroups in the analyses.

In the second experiment, offspring larvae of fem#2, were, at the onset of their last instar, divided into two groups of 21: group (f) that was provided with mature but green, open pods of *C. pallida* and group (g) that was provided with similar closed pods. The other aspects of the experiment were similar to those outlined above for Experiment 1.

In the third experiment, the larvae were collected on 24 November 2014 together with pods of *C. pallida* from the population in north Gainesville that feeds exclusively on this *Crotalaria* species. Larvae were divided into two groups: group (h) was provided with open pods of *C. pallida* that were collected simultaneously with larvae, and group (i) was provided with similar closed pods.

RESULTS

Experiment 1: Influence of seed accessibility of *C. pumila* and *C. spectabilis* on development of *U. ornatrix*

The summary of results for Experiment 1 can be found in Tables 1-2 and Fig. 1. After 11 days of the experiment, difference in larval sizes were already quite obvious between trials. For instance, larvae from group (a) feeding on closed *C. pumila* pods had a mean weight of 0.20 ± 0.05 g - almost twice as light as those from group (d) feeding on closed *C. spectabilis* pods with a mean weight of 0.37 ± 0.02 g (p=0.0003 paired T-test). Group (a) was also lagging behind group (b) that fed on *C. pumila* pods cracked open, which gained 50% more weight (0.31 ± 0.05 gram, p=0.0048 (paired T-test)).

Larvae of *U. ornatrix*, unlike most other Lepidoptera, develop longer if they are male than if they are female, because males tend to have a slightly greater wingspan and they sequester alkaloids used not only in defense, but also in mating (Sourakov, 2015 and references therein). Results are therefore broken down by sex in Fig. 1 and Table 1. Overall, larvae provided with *C. pumila* open pods as diet - group (a) - showed significantly (p=0.03) shorter development time (26.2 ± 2.9 days (n=15)) and greater (p= 0.008 (ANOVA)) FW length
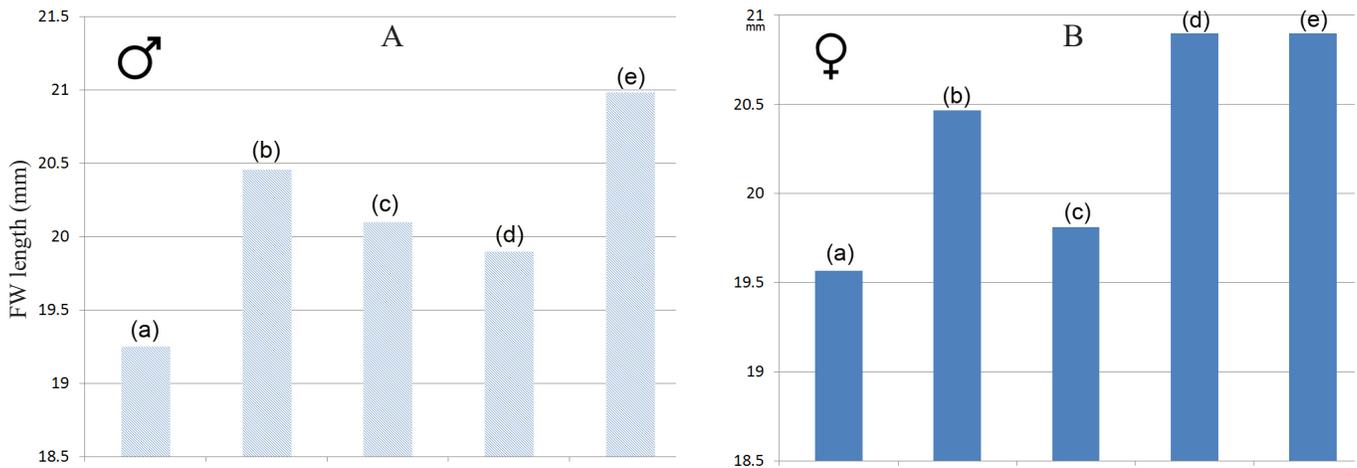
Fig. 1. Influence of accessibility of seeds (open vs. closed pods of *Crotalaria pumila* and *Crotalaria spectabilis* as larval diet) on the size of the resultant adult moths (FW length (mm)). (A) Males (B) Females. (See Tables 1 and 2 for Standard Deviation and P values). (Legend: (a) *C. pumila,* closed pods; (b) *C. pumila,* open pods; (d) *C. spectabilis,* closed pods; (e) *C. spectabilis,* open pods).

Table 1: Influence of accessibility of seeds (open vs. closed pods of *Crotalaria pumila* and *Crotalaria spectabilis* as larval diet) on the size of the resultant adult moths and length of larval development from onset of experiment to pupation.

Diet	*pumila* closed group (a)		*pumila* open group (b)		*pumila* leaves group (c)		*spectabilis* closed group (d)		*spectabilis* open group (e)	
Sex	male	female	male	female	male	female	male	female	male	female
Forewing length (mm)	19.3	19.6	20.5	20.5	20.1	19.8	19.9	20.9	21.0	20.9
Standard Deviation	1.2	1.0	0.9	0.9	0.6	0.5	0.5	0.8	0.8	0.7
Sample size (N, number of individuals)	8	6	9	6	5	9	5	5	6	9
Dev-ment time fr. onset of experiment	30.1 ± 5.6 days		26.2 ± 2.9 days		26.8 ± 2.9 days		25.8 ± 1.9 days		24.5 ± 2.1 days	

Table 2: Pairwise P values for the paired T-test of differences in forewing length of adult moths resulting from feeding in different experimental groups of Experiment 1. The mean forewing values are shown in Fig. 1 and Table 1.

		C. pumila closed		*C. pumila* open		*C. pumila* leaves		*C. spectabilis* closed		*C. spectabilis* open	
		male	female	male	female	male	female	male	female	male	female
C. pumila closed	male	X	0.6	**0.03**	0.1	0.2	0.2	**0.005**	**0.02**	**0.008**	**0.004**
	female	X	X	**0.04**	0.1	0.08	0.2	0.5	**0.02**	**0.03**	**0.02**
C. pumila open	male	X	X	X	1	0.4	0.07	0.2	0.4	0.3	0.4
	female	X	X	X	X	0.4	1	0.2	0.5	0.3	0.4
C. pumila leaves	male	X	X	X	X	X	0.3	0.3	0.1	0.09	**0.004**
	female	X	X	X	X	X	X	0.7	**0.006**	**0.007**	**0.004**
C. spectabilis closed	male	X	X	X	X	X	X	X	**0.04**	**0.05**	0.07
	female	X	X	X	X	X	X	X	X	0.8	0.9
C. spectabilis open	male	X	X	X	X	X	X	X	X	X	0.8
	female	X	X	X	X	X	X	X	X	X	X

Table 3: Outcomes of feeding tests, Experiment 2: larvae of *Utetheisa ornatrix* on open and closed pods of *C. pallida*.

	Open pods group (f)	Closed pods group (g)
Number and % of dead larvae	3 (14.3%)	15 (71.4%)
Total larvae that survived	18 (85.7%)	6 (28.6%)
Larvae unable to penetrate pods	N/A	15 (71.4)
Larvae able to penetrate pods	N/A	2 (9.5%)
Larvae that pupated w/out entering pod	N/A	4 (19%)

Table 4: Outcomes of feeding tests, Experiment 3: field-collected larvae of *Utetheisa ornatrix* on open and closed pods of *C. pallida*.

	Open pods group (h)	Closed pods group (i)
Number and % of dead larvae	3 (23.1%)	6 (37.5%)
Total larvae that survived	10 (76.9%)	10 (62.5%)
Larvae unable to penetrate pods	N/A	9 (50%)
Larvae able to penetrate pods	N/A	4 (25%)
Larvae that pupated without entering pod	N/A	5 (31.3 %)

(20.5 ± 0.8 mm) than larvae provided with closed pods - group (b) - (30.1 ± 5.6 days; n=14; 19.4 ± 1.1 mm).

In contrast, the mean development time (from the onset of the experiment to pupation) for the larvae that fed on open *C. spectabilis* pods - group (e) - was 24.53 ± 2.1 days (n=15) - similar to that of larvae that fed on closed pods - group (d) -(25.8 ± 1.93 days; n=10) (p=0.16 (ANOVA)). The average adult size for the larvae that fed on open *C. spectabilis* pods was 20.93 ± 0.743 mm – similar to that of adults whose larvae fed on closed pods (20.38 ± 0.807 mm) (p= 0.08 (ANOVA)), even though males in group (e) appear a bit smaller (Fig. 1(A)).

We also raised a group of larvae on *C. pumila* leaves – group (c), which we compared to both the *C. pumila* open- and closed-pod groups. The mean development time for these larvae was 26.8 ± 2.8 days, which is closer to the open-pod group, whereas the mean FW length was 19.914 ± 0.52 mm, which is closer to the closed-pod group.

Experiments 2 and 3: Influence of seed accessibility of *Crotalaria pallida* on development of *Utetheisa ornatrix*

Many larvae provided with closed *C. pallida* pods died, being unable to penetrate them (Tables 3 and 4), and a few developed into small adults by scraping the epidermal surface of pods without reaching the seeds. In total, only six out of 39 larvae (15%) provided closed pods of *C. pallida* were able to break through their pericarps. In the Experiment 2, there is no statistically significant difference between mean development times (p=0.6) of the groups provided open and closed pods (18.83 ± 2.33 days, n=18, vs. 19.667± 6.62 days, n=6,

respectively), largerly due to large variation in the closed-pod group (g). The mean size of resultant adults whose larvae fed on open pods - group (f) - was markedly greater than those given closed pods (p= 0.001 (paired T-test)): 19.678 ± .812 mm vs. 17.67 ± 1.88 mm, respectively, because two-thirds of the group (g) pupated without reaching the seeds.

For the same reason, in Experiment 3, adults raised on the open pods - group (h) - were larger (p=0.004 (paired T-test)), with mean FW length of 19.9 ± 0.7 mm vs. 18.5 ± 1.6 mm, while the mean development time from the onset of the experiment to pupation was similar (p=0.7) on open and closed-pod diets (19 ± 1.76 days (n=10) vs. 19.33 ± 1.87 days; n=9).

Mortality in Experiment 3 was not as great as in Experiment 2 because the larvae were older at the start, and hence either were able to penetrate the pods, or pupated prematurely without penetrating any pods. Also, larvae in Experiment 3 came from a population associated with *C. pallida* and might be better adapted for penetration of pods. The most important outcome of the above two experiments lies in the fact that so many larvae (85%) were unable to reach the resource of *C. pallida* seeds due to the protection of the pericarps.

Evaluation of the mechanical properties of the pericarps

The pericarps of the three *Crotalaria* species have approximate sizes as follows: *C. spectabilis* 40 x 10 x 15 mm; *C. pallida* 40 x 6 x 8 mm; *C. pumila* 12 x 6 x 5 mm. While intraspecific variation in size of the pods is not great, one can see from these measurements and photos in Fig. 2 that the interspecific variation is significant and so is the amount of
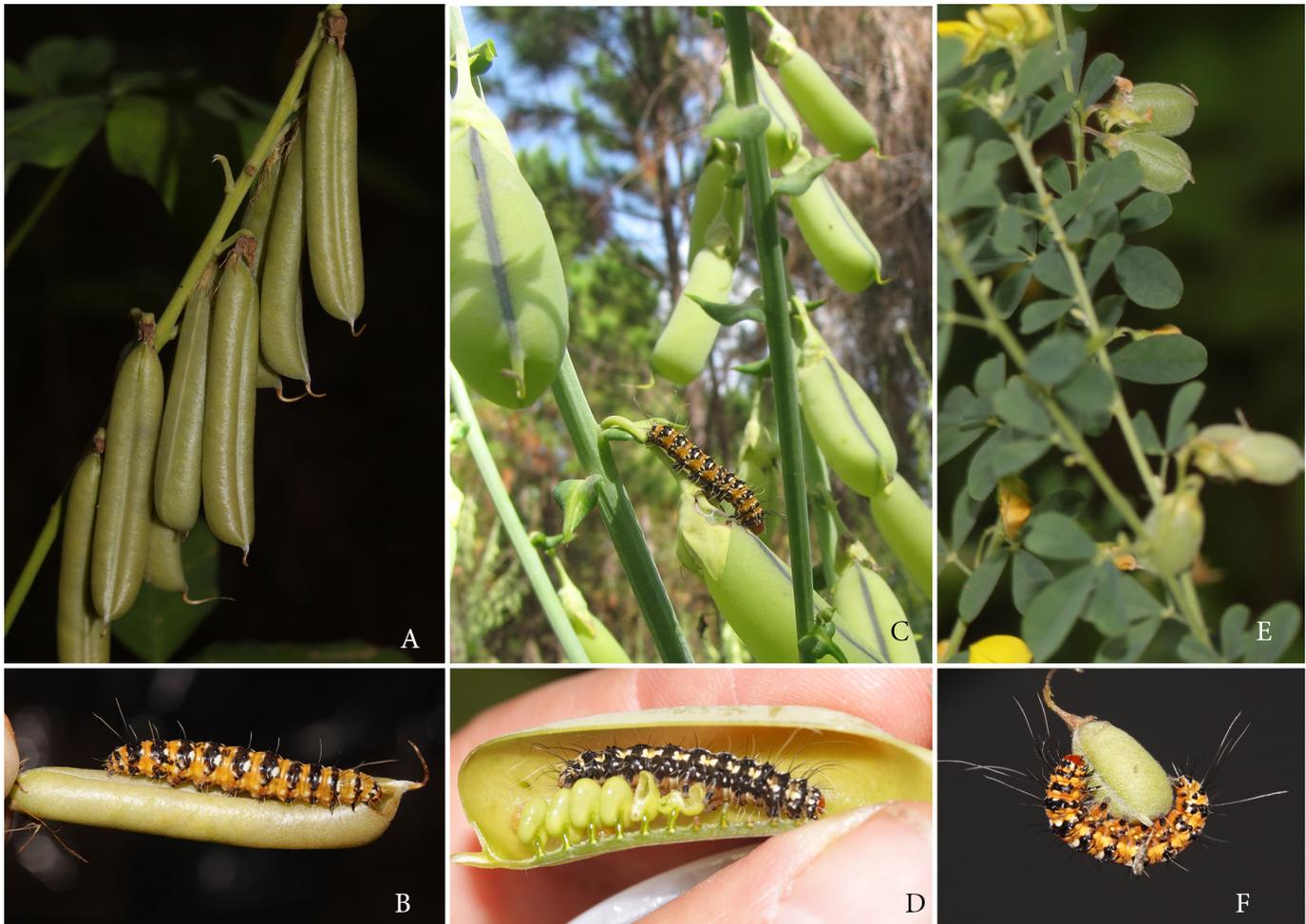
Fig. 2. Caterpillars of the Ornate Bella Moth, *Utetheisa ornatrix* (Erebidae, Arctiinae) attack pods of different *Crotalaria* species to access seeds. The host plants vary in size, number, size and arrangement of pods, and in seed production: (A, B) *C. pallida*; (C, D) *C. spectabilis*; (E, F) *C. pumila*. (photos ©A. Sourakov).

resource obtained by a larva that penetrates a pod. To better understand the mechanism by which different pericarps provide different protection from larvae, we conducted several simple tests of their mechanical properties and also examined them under the microscope. While the most obvious hypothesis would be differences in pericarp thickness, this was immediately rejected upon examining them: the walls of the pericarp are equally thick in all three species.

Thus, the main difference apparently lies in their toughness. As can be observed in a series of photographs of larva chewing through the pericarp of *C. rotundifolia* (in Fig. 3), these pericarps consist of two layers: the soft epidermal layer through which the larva chews relatively easily, and the deeper "woody" layer, working through which costs the larva a significant amount of energy. Under a light microscope, one can see that this latter layer in *C. pallida* and *C. pumila* contains cellulose fibers (Figs. 4A,C). In contrast, in the soft pericarps of *C. spectabilis*, this "woody" layer is not apparent (Fig. 4B).

Using a thumbtack attached to a weight and multiple additional weights, we conducted a pressure-point test, measuring how much force is needed for a needle to penetrate the pericarps. As expected, it required three times less force to puncture *C. spectabilis*, while, surprisingly, *C. pallida* and

C. pumila required the same force. What then accounts for the differences in pericarp defensive ability between *C. pallida* and *C. pumila*? A larva chewing through a pericarp uses a cutting/scraping approach rather than puncturing, and we therefore hypothesized that the difference must lie in the relative strength of the cellulose filaments. We conducted a cutting test, in which a blade of a fine-toothed metal-cutting saw was run the whole length back and forth over the same area (5mm) of pericarps without any pressure but the weight of the blade. We found that it takes twice as many cutting motions to cut through the pericarp of *C. pallida* than the other two species, supporting our hypothesis.

DISCUSSION

Eating through the pericarps of *Crotalaria* may require a great amount of energy from *Utetheisa ornatrix* larvae, as illustrated in Fig. 3 and the associated video. Our experiments suggest that larvae that had to expend energy to penetrate pods of the native *C. pumila* would be at an obvious evolutionary disadvantage over the ones that did not have to expend that energy. The observed difference of four days in development time would give larvae that had free access to seeds a chance
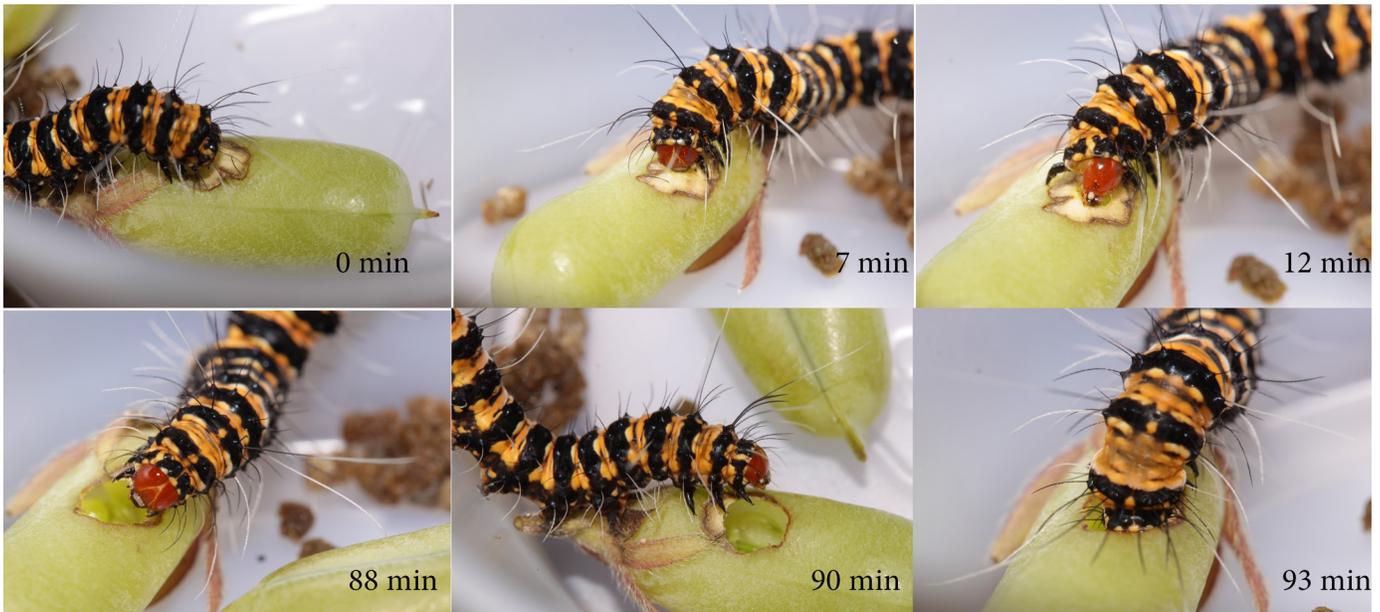
Fig. 3. Caterpillar of the Ornate Bella Moth, *Utetheisa ornatrix* (Erebidae, Arctiinae) chewing through a pericarp of *Crotalaria rotundifolia* to access seeds. It took about one hour of constant chewing before the larva accessed the seeds. (photos © A. Sourakov). Also, see video of caterpillar chewing through the pod at https://www.youtube.com/watch?v=uA0BuWzqxOc

to not only go through more generations faster, but also to occupy host plants earlier than their competitors. Considering that these larvae are cannibalistic when it comes to eggs and younger larvae of their conspecifics, being able to occupy the resource earlier can make a difference between life and death for the whole brood. Hence this, and the fact that larvae that fed on closed pods suffered resulting smaller adult size, also linked to reduced fitness, suggests that the defenses by the pericarps in *C. pumila* are effective deterrents even if the pods were readily available in large clusters. In nature, however, the pods of *C. pumila* are harder to locate as they don't grow in clusters, but are spread out on the plant, so crawling on and between plants becomes necessary. A similar apparent herbivore-avoidance strategy is found in the other native host plant, *C. rotundifolia*.
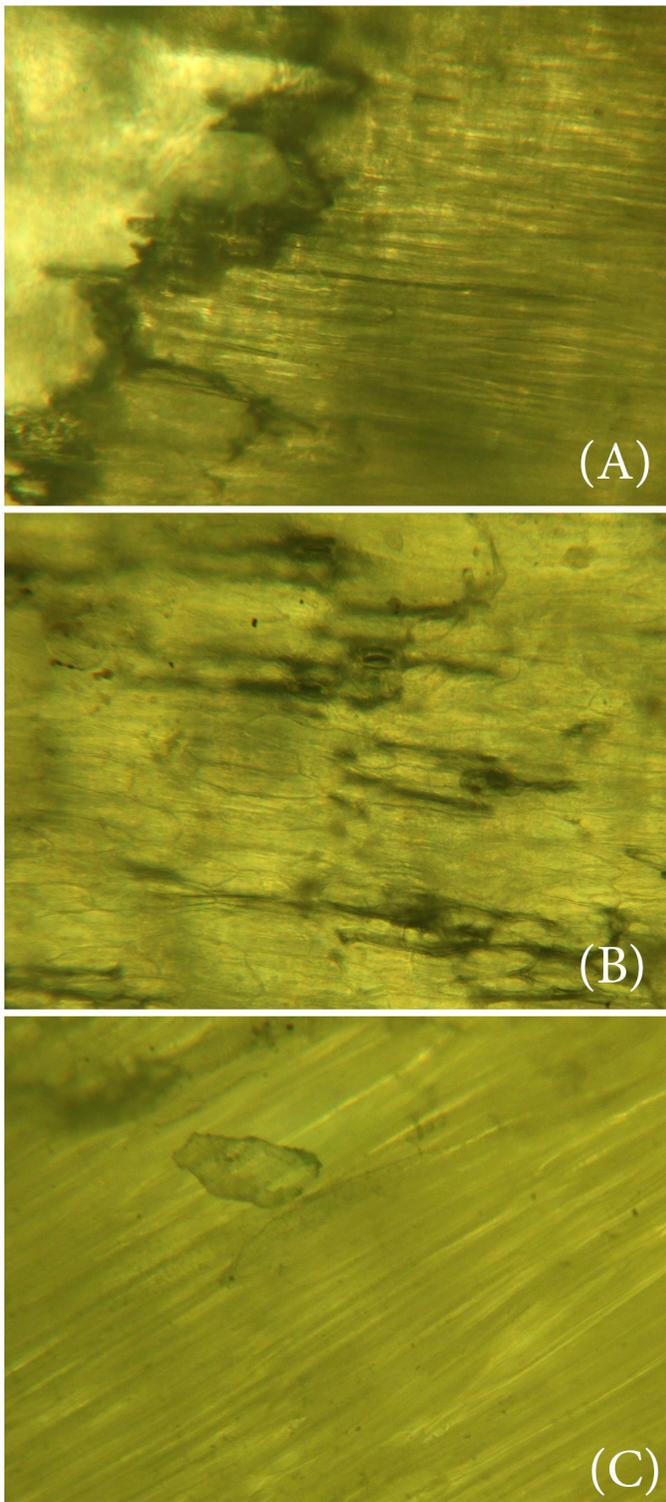
In comparison to the exotic species, the native ones have much smaller pods (Figs. 2 and 3). Differences in dry weight of the seeds suggests that a larva only needs to penetrate a single *C. spectabilis* pod to consume the same amount of food as it would get from breaking into seven pods of *C. pumila* (pers. obs.). Reduction in the number of seeds and their size reduces award a larva receives for penetrating a pod and therefore also may be perceived as a herbivore-avoidance strategy.

Defenses provided by the pericarps of *C. pallida* are even more dramatic: only 15% of the larvae in our tests were able to penetrate pods of this African species. The remarkable toughness of these pericarps is consistent with predictions of the evolutionary arms race hypothesis, suggesting that the longer plants co-exist with specialist herbivores, the better mechanical defenses they possess. While *Utetheisa* may indeed not be the main seed predator of *Crotalaria* in Africa, the fact that there are larger and more ancestrally placed tiger moths with similar biology found on that continent, such as the genus *Amphicallia*, suggests that *Crotalaria* possibly adapted quite early in its evolutionary history to prevent these herbivores

from consuming all their reproductive organs. Based on our knowledge of the evolution of the *Crotalaria*-feeding tiger moths (DaCosta & Weller, 2005; Da Costa, 2010), it is likely that *Crotalaria* started diverging in Africa from its non-toxic ancestors by producing PAs to defend against herbivores such as the tiger moth *Amphicallia bellatrix* (Dalman, 1823), and later diversified into the Asian and New World tropics, where their strategies for evading herbivore pressures have also diverged. This hypothesis is supported by the current understanding of phylogenetic history of *Crotalaria* (Le Roux *et al.*, 2013: 962), which suggests that the ancestral lineages of *Crotalaria* are represented by African species. Both of the exotic species tested here are very successful in their new home in Florida, but *C. pallida* seems to be more widespread. In addition to its tolerance of prolonged and variable seasons, what accounts for its success is its ability to produce numerous seeds, and to defend them, at least partially, from herbivores. In addition to tougher pericarps, these defenses include extra-floral nectaries which attract ants that defend pods, and the sheer number of seeds, with up to 2000 seeds on a single plant.

While *U. ornatrix* has presumably not had sufficient time to fully adapt to the advanced mechanical defenses of *C. pallida* pericarps, the moth still uses this resource effectively. This paradox lies in the fact that during the early stages of pod development, the larvae penetrate them with ease, as the pericarps remain quite soft until seeds fully develop. While seeds at this stage are small and watery, and provide only a limited food resource, *U. ornatrix* larvae take full advantage of the opportunity to get inside the pods and consume them. That explains why empty pods, the seeds of which have been consumed by the larvae, can be frequently found in nature.

The ecological relationships between native *Crotalaria* and *U. ornatrix* are fine-tuned; for example, in sand-hill habitats of north Florida, the adult moths may be found emerging in

soaked sufficiently by the rains. They continue to bloom and produce pods all the way into December, providing plenty of opportunities for *U. ornatrix* to go through multiple generations. Nevertheless, in nature in late fall, when the numbers of larvae are highest, the defense provided to maturing seeds by the pericarps becomes crucial and effective (pers. obs.).

The pericarps of the Asian *C. spectabilis* provided only a limited protection from larvae in our experiments. While not significantly slowing down larval development, the penetration of the pericarps led to smaller male adults, which has been shown to reduce their fitness. These defenses fall short of those produced by *C. pumila* and *C. pallida*. However, much more work, which is beyond the scope of the present study, is necessary to test the evolutionary arms race hypothesis. Such a project would involve a thorough examination of biology and evolutionary history of many *Crotalaria* species and associated herbivores across the globe, and would be of great value for our understanding of host-herbivore co-evolution.

The New Associations Hypothesis (Hokkanen & Pimentel 1989) proposes another perspective: an introduced species will not fare as well as a native species against attack due to a lack of shared evolutionary history. Cogni (2010) found support for this hypothesis. Our results suggest that the introduced species with the presumed longest history of co-evolution, *C. pallida*, showed the best defense against predation as measured by the highest larval mortality rates, whereas, another introduced species, *C. spectabilis*, was less successful in its defense. Our results do not contradict the theory behind the New Associations Hypothesis, namely that plants that have evolved with herbivores a longer period of time are better protected, but they also illustrate that simple generalizations (i.e., introduced species will do worse than native species) will not always be correct.

ACKNOWLEDGEMENTS

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Fig. 4. Light microscope images of the pericarp wall showing differences in the presence and structure of cellulose fibers that prevent *Utetheisa ornatrix* larvae from penetrating the pods: (A) *Crotalaria pallida*; (B) *C. spectabilis*; (C) *C. pumila*.

the spring as *C. rotundifolia* plants sprout from their roots and, by the time the larvae are able to penetrate the pods, the pods begin to harden, with both species racing against each other in development. *Crotalaria pallida*, being a novel host, sprouts much later in the season from seeds after the latter are

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