Journal of Young Investigators RESEARCH ARTICLE

The Effect of Food Type on the Development rate of the Danaid Eggfly

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Butterflies are important components of community trophic structure because they serve as food for mammals, lizards, birds, other insects, and spiders (Schreiner, 1997). Furthermore, certain species of butterfly are also valued pollinators and herbivores; therefore, butterflies are important in maintaining ecosystem biodiversity (Hammer, 2014). Individual butterflies are vulnerable as larvae because they are small and slow. They are also immobile as pupae or cocoons. A shortened larval and/or pupal period should decrease the chances of being ingested or attacked by predators and increase the probability of surviving to adulthood. Therefore, efforts to identify food types effective at increasing the development rates of threatened butterfly species may be useful. The purpose of this study was to determine whether different food types affect the rate of development of *Hypolimnas misippus*, also known as the Danaid eggfly. Two experimental groups with twenty larvae each were used in the study. One half was fed *Portulaca oleracea* and the second half was fed *Portulaca quadrifida*, both of which are common food plants for *H. misippus.* To determine if growth rate depended on food type, the number of days from egg to pupa, the pupal weight, and the number of days from pupa to adult were recorded. Short period of days between stages and a high pupal weight would indicate a fast growth rate. The findings revealed a difference in the larval period between the two groups ($p=1.0 \times 10^{-5}$) with the *P. oleracea* group having a shorter larval period in comparison to the *P. quadrifida* group. However, there was no difference in the pupal weight and pupal period between the two groups ($p=0.684$ and $p=0.199$ respectively). These results suggest *P. oleracea* may increase the development rate, specifically the larval period of *H. misippus.* This would allow this species of butterfly to reach adulthood faster and increase their population size.

INTRODUCTION

There are 860 different species of butterflies in Ghana. One such species is the *Hypolimnas misippus* or the Danaid eggfly (Gordon, 2010). *H. misippus* eats a variety of different food plants from several plant families such as Convolvulaceae, Malvaceae, and Portulacaceae (Mensah and Kudom, 2011). The primary food type of *H. misippus* is *Portulaca oleracea*, which belongs to the Portulacaceae family; however, *H. misippus* is also known to consume *Portulaca quadrifida*, another member of the Portulacaceae family (Mensah and Kudom, 2011).

The *H. misippus* species was specifically chosen for this study because females have had no problems laying eggs in research settings. Also, the species appears to be in decline since June 2009 in some areas in Cape Coast, Ghana as indicated by Mensah and Kudom (2011). The species still remained to be scarce as this study was conducted during the summer of 2012. It is also important to note a species survey is to be conducted at a later date to determine if *H. misippus* is endangered. In the meantime, however, the concept and results of this study will still benefit future butterfly conservation research.

Efforts should be made to conserve *H. misippus* or butterflies in general because they are critical players in maintaining biodiversity (Schreiner, 1997). An example of a specific predator of *H. misippus* is *Lanius collaris*, a fiscal shrike, which was observed to feed on larvae in a prior study conducted by Mensah. It can be predicted that if the population of *H. misippus* declines, then it is likely the population of fiscal shrikes could also decline as a consequence.

Schreiner (1997) describes the four stages of the life cycle of a butterfly: egg, larva, pupa, and adult. First, the adult butterfly lays its eggs on the appropriate host plant. Then, the eggs hatch and small larvae emerge. The larval stage is subdivided into different phases called instars, and as the instar stage progresses, the larvae grow larger (Benrey and Denno, 1997). Subsequently, after sufficient food is obtained and converted into energy, the larvae then transform into pupae. Inside the pupae, the larva form disintegrates and reorganizes itself into the adult form. After about a week, the pupa opens and the butterfly emerges (Schreiner, 1997).

The goal of this study was to determine if a particular food type affected the growth rate of *H. misippus*. If a food type positively affected the growth rate of the Danaid eggfly, then researchers would need to work towards conserving that particular species of plant. By having a faster growth rate, *H. misippus* would be able to avoid being eaten and being attacked by predators at such early stages of development, therefore having a higher probability of surviving to adulthood when they can escape predators easily by utilizing flight.

Conversely, according to the slow-growth, high-mortality hypothesis, individuals that feed on a poor quality food would develop slowly and would therefore have a larger window of vulnerability to predation and thus an increased risk of mortality (Cornelissen and Stiling, 2012). In a similar study, researchers varied the development rates of butterfly larvae by rearing

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individuals on different food plants (with variations in the amount of protein) and also by altering the temperature conditions, both of which affect the development rate (Benrey and Denno, 1997). It was observed that larvae with fast development rates had fewer incidents of parasitism than larvae with slow development rates (Benrey and Denno, 1997). This was because the window of vulnerability to predation or parasitism was much smaller for larvae with faster development rates than larvae with slow development rates (Benrey and Denno, 1997).

In a more recent study conducted by Mensah and Kudom (2011), they studied whether different food plants, *P. oleracea*, *P. quadrifida*, and *Asystasia gangetica,* in the Cape Coast area affected the development rate of *H. misippus* (Mensah and Kudom, 2011). The number of days from egg to pupa (larval period), pupal weight, and the number of days from pupa to adult (pupal period) were determined and also the nutritional content of each plant was analyzed (Mensah and Kudom, 2011). A shortened larval and pupal period and heavy pupae would indicate a fast development rate. It was determined that *H. misippus* had the fastest larval development rate and heaviest pupae when fed *P. oleracea*, but similar pupal development rates when fed on any of the three plants (Mensah and Kudom, 2011). Other similar studies involving different food types affecting the development rate of *H. misippus* were difficult to find.

In this study, two food plants from the Portulacaceae family were focused on to distinguish whether there is a difference in the species of Portulacaceae itself (*P. oleracea* versus *P. quadrifida*) on the development rate of *H. misippus*. In addition to this, food intake was focused on, rather than nutritional content, to determine whether the species had a preference for either food type. This could be analyzed by determining the food type that is replaced more often as a result of higher consumption by larvae. Lastly, this study contributes to the very few studies looking at whether specific food type affects the development rate of *H. misippus* (Gordon, 2010).

MATERIALS AND METHODS

Catching *Hypolimnas misippus*

The first female specimen, assumed to be mated, was caught at the Botanical gardens of the University of Cape Coast (UCC) on May 23rd, 2012. The butterfly was caught with an aerial net and then placed in a ventilated transport container to bring to the lab. However, the first female specimen did not lay eggs. Another six days elapsed until another specimen, also assumed to be mated, was caught at the Research farm of UCC. Fortunately, after three days this subject laid eggs on June 3rd, 2012.

Preparing for the study

A small metal tin that contained handfuls of *P. quadrifida,* (the host plant where Oviposition or egg laying would occur), was placed on top of brown paper. Next, a large square box with mesh siding was then placed on top of the tin containing the plant material (to house the female specimen). The adult female specimen was then taken out of its ventilated transport container and released into the box with the mesh siding. A heat lamp, to simulate sunlight (*H. misippus* is active during the day), was placed adjacent to this set-up and pointed down towards the tin containing the *P. quadrifida*. The female was taken out of the mesh box daily to be fed with 10% (weight/volume) sucrose sugar solution.

Experimental Design

Once the eggs hatched into small black larvae, the box apparatus was removed. The tin of *P. quadrifida*, a large magnifying glass, a paint brush, 50 plastic cups, large amounts of mesh, and filter paper were transferred to the UCC lab (for better lighting to transfer the larvae to the cups). Once a larva was identified with the magnifying glass, it was then transferred carefully with the paintbrush. 25 larvae were chosen haphazardly and were fed *P. oleracea* (20 in the experimental group, 5 in the extra or back-up group). A second set of 25 larvae were chosen haphazardly and fed *P. quadrifida* (20 in the experimental group, 5 in the extra or back-up group). Each cup was approximately 200mL and contained a filter paper (for waste collection) at the bottom along with a handful of food plant. The cup was covered with mesh and was rubber banded closed. Food plant was not weighed before placing in each cup because the subjects were fed *ad libitum*. Thus, large quantities or handfuls of food were placed in the cups daily and food was given more often as the larvae changed instars.

There were 25 replications for each food plant. All cups were placed together in the UCC lab under a constant temperature of 26ºC with a lighting of 12L:12D. Therefore, temperature was not a confounding factor and would have not affected the development rate. In sum, the experimental and extra groups were fed for a maximum of 18 days.

Materials such as filter paper, tweezers, rubber bands, napkins, a petri dish (with a cover) and a paintbrush were gathered to replace food every morning. First, each larva was removed from their cup by brushing them with a paintbrush into a petri dish. The dish was then covered to prevent escape. Next, any leftover old food and old filter paper were removed and thrown away. The cup was cleaned with a napkin, the filter paper was replaced and set on the bottom of the cup, and then a handful of fresh food plant was placed back into the cup along with the larva. Mesh was placed back on the cup, rubber banded and then placed back in the experimental area in the lab.

Once the larvae pupated (larvae attached to the top of the mesh to form a cocoon), each pupa was removed from the mesh of the cup (with tweezers) and placed at the bottom of their own larger cup on top of a napkin. Then each cup, approximately 380mL was labeled, covered with mesh, rubber banded, and set aside. Every morning new pupae were weighed and recorded. Once they become adults, they were fed with 10% sucrose sugar solution (w/v). Some adults were kept for a future study, while others were set free.

Means and standard deviations (excluding pupal weight measurements) were rounded to the nearest tenth or whole number. Statistical analysis was performed using a Mann-

Whitney U test. A p-value of less than 0.05 was considered to be significant.

RESULTS

All eggs hatched on June 3rd, 2012, the last individual pupated on June 20th, and the last adult emerged from its pupa on June 27th. The larval group fed *P. oleracea* group included nine females and eleven males emerged. Ten females and ten males were fed *P. quadrifida* during the larval period.

Mean number of days from egg to pupa

For larvae (n=20) that were fed *P. oleracea*, the range to pupate was between 12 and 15 days (Figure 1). For larvae (n=20) that were fed *P. quadrifida* the range to pupate was between 13 and 17 days. Larvae fed *P. oleracea* spent less time in instars 2 to 4, while larvae fed *P. quadrifida* only spent less time in instar 1 (Table 1). The mean number of days to pupate for larvae fed *P. oleracea* was 13 ±1 days (12.85 ±0.93 days) (Figure 2). For larvae fed *P. quadrifida* the mean was 15 ± 1.5 days (15.1±1.4 days). It was determined that the difference in populations is statistically significant ($p=1.5 \times 10^{-5}$) (Figure 2).

Mean pupal weight

For larvae fed *P. oleracea*, the range was between 0.50 g and 0.88 g (Table 2). For larvae fed *P. quadrifida* the range was between 0.49 g and 0.73 g. The mean pupal weight for the larvae fed *P. oleracea* was 0.62 ± 0.09 g (Figure 3). For larvae fed *P. quadrifida*, the mean was 0.62± 0.089 g. It was determined that the difference in populations is not statistically significant (p=0.684) (Figure 3).

Mean number of days from pupa to adult

For larvae fed *Portulaca oleracea*, the range was between 6 and 8 days for adults to emerge from the pupae (Figure 4). For larvae fed *P. quadrifida*, the range was between 6 and 8 days for adults to emerge. The mean number of days for adult emergence for larvae fed *P. oleracea* was 7 ± 0.5 days (7.05 ±0.39 days) (Figure 5). For larvae fed *P. quadrifida*, the mean was 7 ± 0.5 days (6.85 ±0.58 days). The difference in populations is not statistically significant (p=0.199) (Figure 5).

Table 1. Mean Number of Days of instar stages of Danaid eggfly larvae fed *Portulaca oleracea* versus *Portulaca quadrifida* (± s.d.).

	P .oleracea	*P. quadrifida*
Instar 1	1±1 day (1.2±0.6 days)	1 day
Instar 2	4±1 day (4.2±0.7 days)	5± 1 day(4.55±0.7 days)
Instar 3	3±1 day (3.2±0.6 days)	4±1 day (4.4 ±0.9 days)
Instar 4	4±1 day (4.2±1 day)	5±1 day (5.1 ±1.0 days)

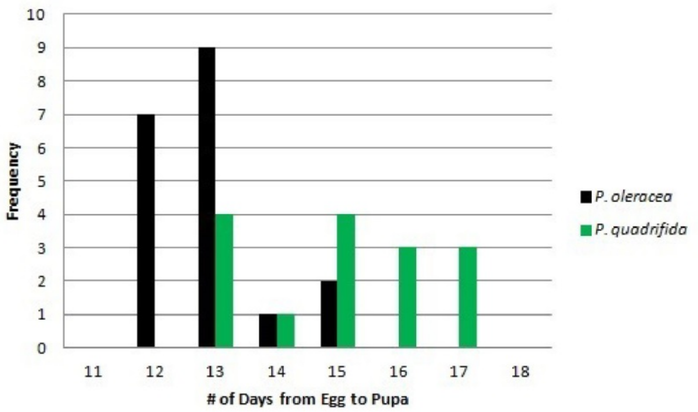


Figure 1. Number of Days from Egg to Pupa of Danaid eggfly larvae fed *Portulaca oleracea* versus *Portulaca quadrifida*. The number of days to pupate (to transform from an egg to a pupa/cocoon) for individuals reared on *P. oleracea* vs. *P. quadrifida*. Those reared on *P. oleracea* spent from 12 to 15 days as larvae, while those reared on *P. quadrifida* spent from 13 to 17 days as larvae. One can infer that those reared on *P. oleracea* spent fewer days as larvae while those reared on *P. quadrifida* spent more time as larvae.

DISCUSSION

Survivorship

A few specimens died due to disturbance during pupation (possibly caused by a mouse). Originally, all 50 specimens were kept in an office, but due to an ant infestation on June 7th 2012, it was decided to move all butterfly larvae to a UCC lab. The disturbances happened on both June 17th and June 20th 2012. For *P. oleracea,* on June 17th an extra specimen from the non-experimental group died and on June 20th one specimen from the experimental group died due to the disturbance (denoted with * in Table 3). For *P. quadrifida,* one specimen on June 20th died due to an excessive leakage from the pupa. This was due to human error when removing the pupa from the mesh of the cup for weighing (denoted with ** in Table 3). It was then decided that data from deceased experimental group larvae would be replaced by living random larvae from the appropriate back-up group

To determine if food type affected survival, only the specimens that died during the larval period (not pupal period since no food is consumed) were considered. One specimen out of 25 larvae (0.04%) from the *P. oleracea* group (experimental or non-experimental) died early in the study, however, three specimens (12%) from the *P. quadrifida* group (experimental or non-experimental) died (Table 3). The difference in survivorship between these two groups could indicate a relationship between food type and survivorship. This should be explored in future studies.

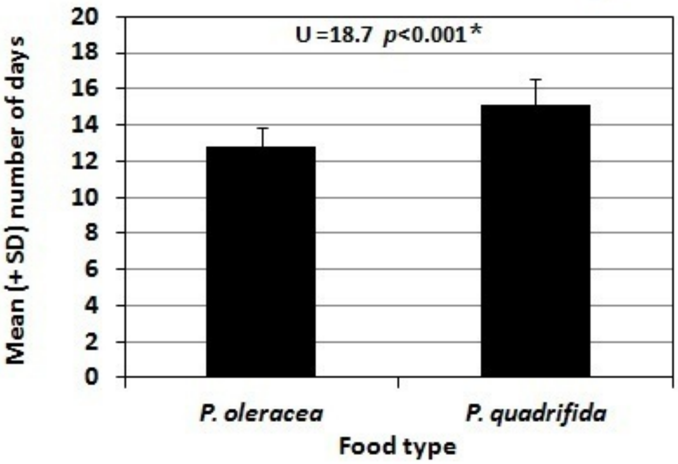


Figure 2. Mean Number of Days from Egg to Pupa of Danaid eggfly larvae fed *Portulaca oleracea* versus *Portulaca quadrifida* (± s.d.). Individuals reared on *P. oleracea* spent an average of 12.85 ±0.93 days as larvae, while individuals reared on *P. quadrifida* spent an average of 15.1±1.4 days as larvae. It was determined that the difference in time spent as larvae between the two populations was statistically significant* ($p=1.5x\ 10^{-5}$).

Larva #	*P. oleracea* Group (g)	*P. quadrifida* Group (g)
1	0.59	0.50
2	0.56	0.53
3	0.57	0.53
4	0.53	0.68
5	0.52	0.67
6	0.59	0.64
7	0.55	0.67
8	0.60	0.50
9	0.70	0.61
10	0.72	0.69
11	0.50	0.72
12	0.88	0.73
13	0.65	0.49
14	0.76	0.66
15	0.67	0.51
16	0.61	0.68
17	0.65	0.56
18	0.64	0.72
19	0.54	0.61
20	0.53	0.73

Table 2. Pupal Weight of Danaid eggfly larvae fed *Portulaca oleracea* versus *Portulaca quadrifida*. The weight of the pupae/cocoons of individuals reared on P. oleracea versus P. quadrifida. For larvae reared on P. oleracea the range was between 0.50 g and 0.88 g, while for larvae reared on P. quadrifida the range was between 0.49 g and 0.73 g.

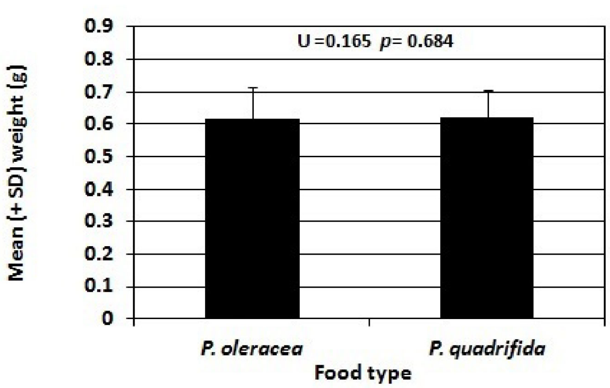
Measuring Food Intake and Waste

Food intake and waste were not measured quantitatively due to time constraints. However, future studies should consider this method to deduce if a food type is of poor quality or good quality. A large amount of food ingested with a large amount of waste expelled would signify a poor quality food source, while a large amount of food ingested and a minimal amount of waste expelled would signify a goody quality food source. Plant species that are identified as good quality food types should therefore be conserved to aid in the development of potentially endangered butterfly species.

Although food intake and waste were not measured in this study, one could still consider the nutritional content of *P. oleracea* versus *P. quadrifida* to help determine its quality. Protein is important during the larval phase as it is stored for use during metamorphosis or pupation (Bauerfiend, 2005). *Portulaca quadrifida* has a protein content of only 19.6g/100grams of plant, while *P. oleracea* has an even lower protein content of only 5mg/100g (Getachew, 2013; Singh, 2011). However, the *H. misippus* larvae may have eaten more of the *P. oleracea* and expelled less, rendering this significant protein difference irrelevant.

Regarding other nutrients, *P. oleracea* does have several beneficial properties. *Portulaca oleracea* has the highest omega-3 acid content compared to other leafy green plants and is also rich in nutrients and minerals such as urea, calcium, iron, phosphorus, manganese, and copper (Rashed, 2004; Dhole, 2011). While, *Portulaca quadrifida* is rich in oxalates, harmful minerals, which can lead to nutritional damage (Getachew, 2013). Therefore, this may explain the preference for *P. oleracea* over *P. quadrifida*. However, future studies should still explore the nutritional content of both plants further.

Figure 3. Mean Pupal Weight of Danaid eggfly larvae fed *Portulaca oleracea* versus *Portulaca quadrifida* (± s.d.). Individuals reared *on P. oleracea* had an average pupal weight of 0.62 ± 0.09 g while those reared on *P. quadrifida* had an average pupal weight of 0.62± 0.089g. It was determined the difference between pupal weights between the two populations was not statistically significant (p=0.684).



Mean number of days from egg to pupa

The results were statistically significant ($p<0.05$). *Portulaca oleracea* may be of better quality than *P. quadrifida* as larvae fed *P. oleracea* had a shorter larval period (Figure 2). Although there was no significant difference in the pupal weights or pupal period, having a shortened larval period would still be beneficial to the survival of *H. misippus* as described in the introduction.

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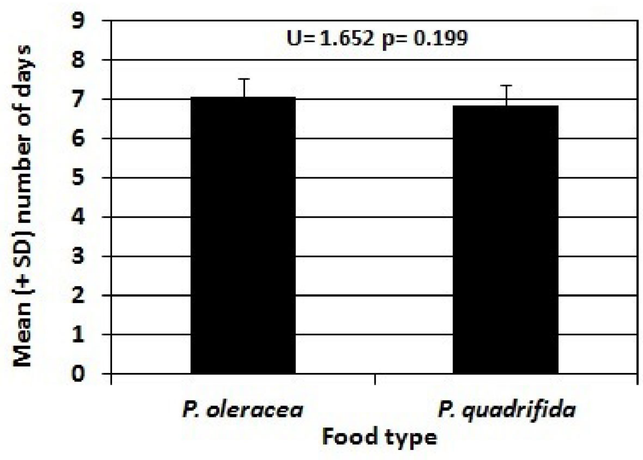
P. oleracea	Date of Death	Time of Death	*P. quadrifida*	Date of Death	Time of Death
Larva 3*	6/20/12	Pupal Period	**Larva 6****	6/20/12	Pupal Period
Larva 10	6/7/12	Larval Period	**Larva 11**	6/9/12	Larval Period
Extra 2*	6/17/12	Pupal Period	**Larva 12**	6/17/12	Larval Period
			Extra 3	6/17/12	Larval Period

Table 3. Survivorship of individuals reared on *Portulaca oleracea* versus *Portulaca quadrifida.* For the *P. oleracea* population three individuals died (3 and 10 from the experimental group and extra 2 from the non-experimental or back-up group). For the *P. quadrifida* population four individuals died (6, 11, and 12 from the experimental group and extra 3 from the non-experimental group). Those that died during the pupal period was due to the disturbance with the mouse* or due to human error** as described in the text. Individuals that died during the larval period were considered to determine the survivorship (regarding food type) between the two populations. It was determined that one specimen out of 25 (0.04%) larvae from the *P. oleracea* population (both experimental and non-experimental) died, while 12% of the specimens from the *P. quadrifida* group died. There may be a correlation between food type and survivorship; this should be analyzed in future studies.

Therefore, scientists should work towards conserving *P. oleracea* to maintain the *H. misippus* population.

Figure 5: Mean Number of Days from Pupa to Adult of Danaid Eggfly larvae fed *Portulaca oleracea* versus *Portulaca quadrifida* (± s.d.). Individuals reared on P. oleracea spent an average of 7.05 ±0.39 days as pupae, while individuals reared on P. quadrifida spent an average of 6.85 ±0.58 days as pupae. It was determined that the difference in time spent as pupae between the two populations was not statistically significant (p=0.199).



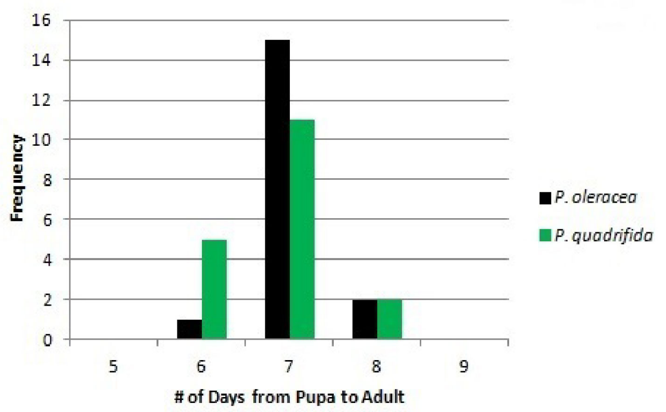


Figure 4: Frequency of Days from Pupa to Adult of Danaid Eggfly larvae fed *Portulaca oleracea* versus *Portulaca quadrifida*. The number of days for adults to emerge from pupae for individuals reared on *P. oleracea* versus *P. quadrifida*. The range was between 6 and 8 days for adults to emerge from the pupae for both populations reared on *P. oleracea* and *P. quadrifida*.

First Generation Mating and Gender of Offspring

In future studies, researchers could determine whether food type affects the fitness of *H. misippus* by mating individuals of the same food type and individuals of different food types together. Researchers should consider observing the number of eggs laid and the survivorship of the offspring. Scientists could also determine whether the development rate of the second generation is different from the development rate of the parental generation. Lastly, researchers could investigate whether the food type affects the sex of the offspring (gender bias).

Conclusion

Unlike its predecessor, this study solely focuses on the Portulacaceae family to determine whether a different species in the same genus could affect the development rate of *H. misippus*. Also, a key feature of this study is that it suggests that the larval period, when food is consumed, is the critical time for the development rate of *H. misippus*. Therefore, since *P. oleracea* did accelerate the development rate of *H. misippus*, then conservation work should be done to preserve this plant species. A faster development rate, or specifically larval period, would allow individuals to develop through this stage faster when they are easily preyed upon. This would allow individuals to reach adulthood sooner. This would allow them to easily escape predators, and consequently would increase the species population. Therefore, this study presents vital information regarding the preservation of the *H. misippus* butterfly; the importance of preserving a plant that enhances its chance of survival.

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