Do hawkmoths (*Hyles lineata*) have an innate preference for plants that produce linalool?

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Introduction

Plant-insect interactions have been implicated as a key driver of plant diversification. In order to thrive, plants are under selection to maximize pollinator attraction while at the same time defend themselves against antagonists as part of an evolutionary arms race. Across a plant's geographic range, its interactions with one species of insect may vary from mutualistic to antagonistic, leading it to develop varied defenses[1]. These contrasting implications on plant fitness may explain the variation in traits across time and space, especially when a plant's key pollinator is also an antagonist.

While the adult white-lined sphinx moth, *Hyles lineata*, is a key pollinator of the Arkansas River Valley evening primrose *Oenothera harringtonii* (Onagraceae), it also lays its eggs on the plant, and the emerging larvae eat the plant tissues[2]. Production of the scent compound linalool varies geographically in *O. harringtonii*, leading to polymorphic populations. While some floral scent compounds have been linked to higher rates of herbivory, others may act as defensive volatiles[3]. These compounds may work as an oviposition stimulant or as an honest signal of other defensive traits. Previous work has found that neonates have higher survivorship on populations that do produce linalool (Broadhead, unpublished data).

Here, we tested the hypothesis that in a polymorphic *O. harringtonii* population, *H. lineata* females and neonates preferentially select plants that produce linalool.

Figure 1. The *O. harringtonii-H. lineata* system (Part 1). From L to R: *O. harringtonii* plant in flower, *H. lineata* adult (pollinator).

Methods

Oviposition Assays

Mated adult females were left overnight in a quonset containing 8 *O. harringtonii* plants from 2 maternal lines. All plants were checked for eggs the following morning.

Choice Assays

First instar neonates (<24 hours old) were given 24 hours to choose between leaf discs from two maternal lines.

Feeding Assays

First instar neonates (<24 hours old) were placed on leaves and allowed to eat for 72-120 hours. Relative growth rate, consumption rates and efficiency of conversion were calculated.

Results

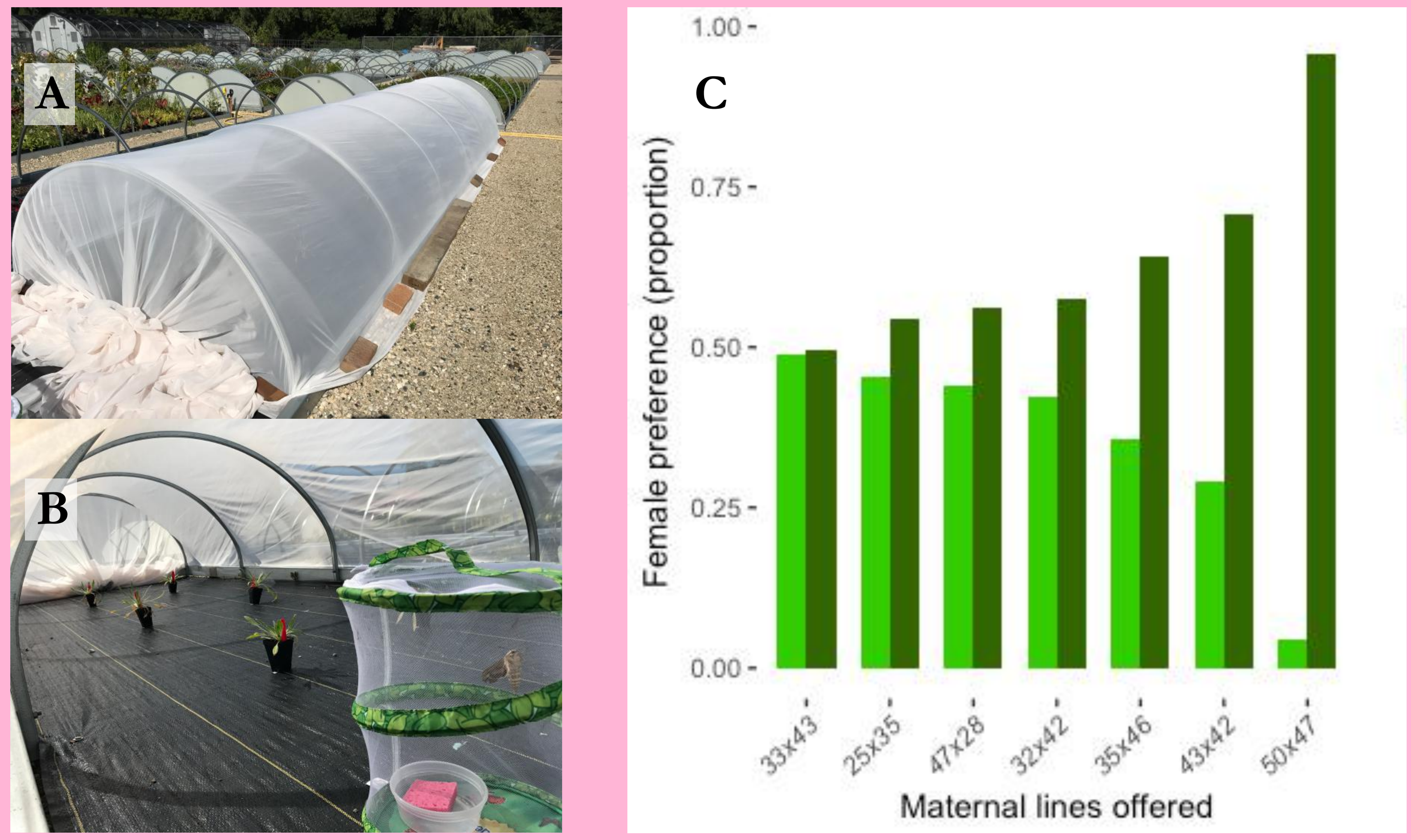

Figure 2. Oviposition assays tested female choice. A) View of the outside of the quonset. B) Experimental set up inside the quonset. C) Proportion of eggs laid on each maternal line. Nine females laid a total of 1,026 eggs. For each assay, light green bar = first maternal line and dark green = the second maternal line.

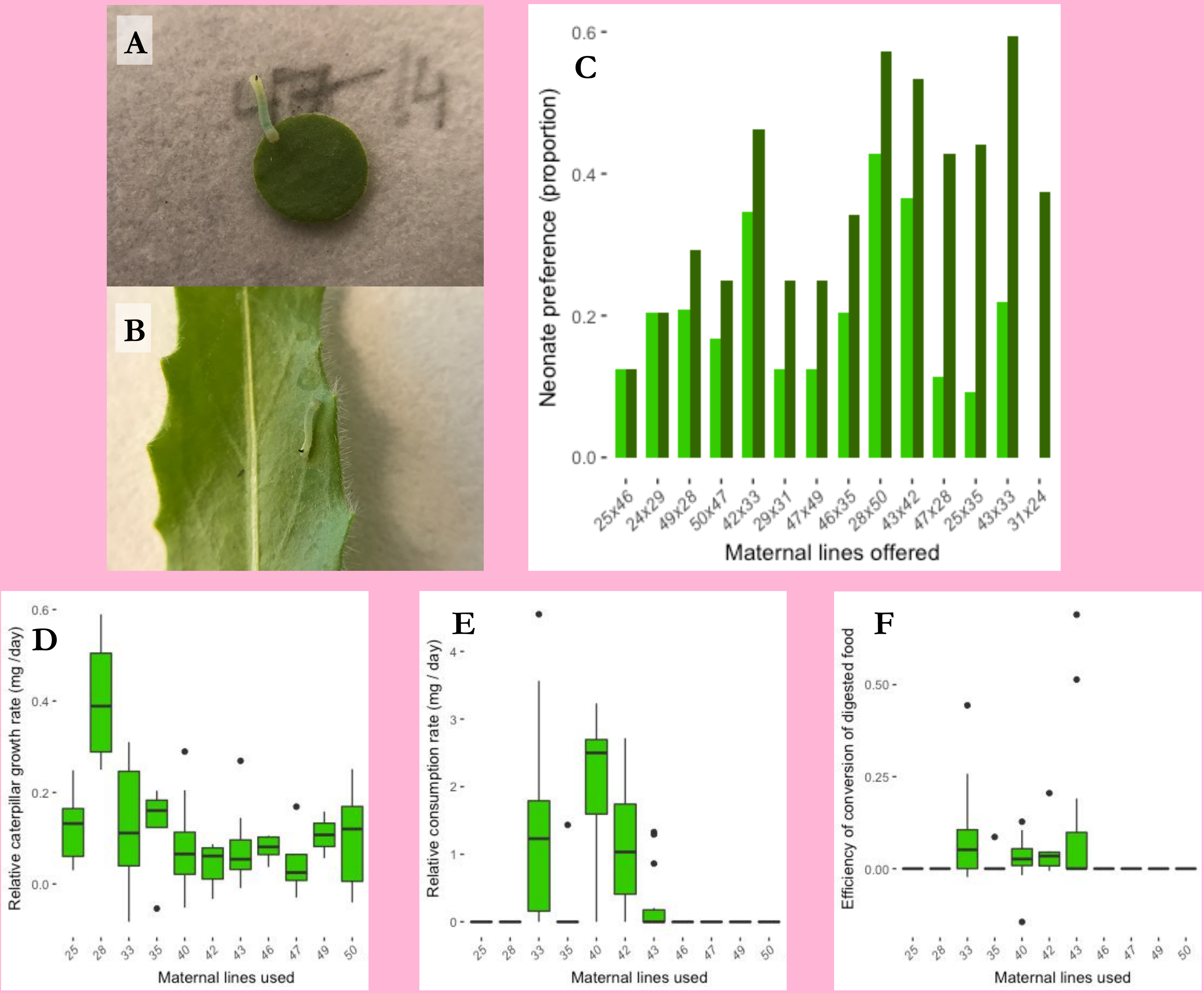

Figure 3. A) Neonate choice assay: neonate on a leaf disc. B) Neonate feeding assay: neonate on a leaf. C) Proportion of neonates who chose each maternal line in choice assays. N=310 total assays, but 42% (N=146) of neonates did not make a choice. For each assay, light green bar = first maternal line and dark green = the second maternal line. D) Relative growth rate of neonates on different maternal lines (overall $p<0.001$). Neonates grew fastest on maternal line 28 ($p<0.001$). E) Relative consumption rate of leaf material (overall $p<0.001$). Neonates ate material from maternal line 40 the fastest ($p<0.001$), followed by maternal lines 33 ($p<0.001$) and 42 ($p<0.05$). F) Efficiency of conversion of digested food (overall $p=0.2281$).

Conclusions

Female hawkmoths strongly preferred maternal lines 47, 42, and 46. Hawkmoth neonates strongly preferred maternal lines 24, 33, and 35, and grew fastest on line 28.

These preferred maternal lines are suspected to produce linalool. Once the *O. harringtonii* plants have bloomed, scent samples will be taken and analyzed to determine linalool production. If our hypothesis is confirmed, we would conclude that *H. lineata* prefer plants that produce linalool. By preferentially ovipositing on and eating linalool-producing plants, *H. lineata* is exerting a negative selective force on that chemotype. Thus, in populations where *H. lineata* is an antagonist, we would expect to see fewer *O. harringtonii* plants producing linalool. Further research would be required to determine if the leaves themselves produce linalool as an attractant or if floral linalool production indicates other attractive traits. It would also be interesting to quantify other defensive traits, such as trichome density, to determine if there is a link between physical defenses and linalool production.

Further research is also necessary to confirm that linalool is a heritable trait, meaning that all siblings in a maternal line have the same chemotype, so hawkmoths should be responding to all siblings in the same manner. Thus, in comparisons where *H. lineata* shows no clear preference for one maternal line over the other, we would expect both maternal lines to have the same chemotype.

Together, these data will help us to understand the role of floral scent and other chemical signals in mediating selection due to plant-insect interactions.

Figure 4. The *O. harringtonii-H. lineata* system (Part 2). From L to R: *H. lineata* eggs, neonate, and caterpillar.

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