Echinacea Project
Research Proposal

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21 June 2011

Plan A: Investigating the interaction between *Echinacea angustifolia* and *Echinacea pallida*

Note: The viability of Plan A depends on the flowering population size of *Echinacea pallida*.

Background

The genus *Echinacea* consists of nine North American species (Flagel et al. 2008). Of these, only *E. pallida* is considered to potentially be a polyploid, having both triploid (2n=33) and tetraploid (2n=44) lines (Mechanda et al. 2004). The exact origin of *E. pallida* is not known. Its alleles are often found among the clade consisting of *E. angustifolia*, *E. atrorubens*, and *E. laevigata*, though it does have similarities with other *Echinacea* clades. This suggests *E. pallida* may have arisen from multiple parental lines, or branched earlier in *Echinacea*'s species differentiations (Flagel et al. 2008).

E. angustifolia is a self-incompatible species through the S allele mechanism (Wagenius et al. 2007). In an experiment last year, pollen from two *E. pallida* individuals was used to pollinate *E. angustifolia*. This pollen caused styles to shrivel in all crosses except for a cross between a specific *E. pallida* individual and a specific *E. angustifolia* individual. The relationship of *E. pallida* and *E. angustifolia* can help to illuminate the age of the self-incompatibility system seen in *E. angustifolia*. The previous experiment leads one to speculate that the self-incompatibility system of *E. angustifolia* may be old enough to be present in multiple *Echinacea* species.

E. pallida may also play a role in the persistence of *E. angustifolia*. In some restorations, including the Hegg Lake Wildlife Management Area in the study area, non-native *E. pallida* was planted instead of the native *E. angustifolia*. *E. angustifolia* is known to be pollen limited (Wagenius 2004). This limitation is not due to a lack of pollinators (Wagenius and Lyon 2010). If *E. pallida* pollen then is able to cause styles to shrivel in *E. angustifolia*, yet not produce viable seeds, this could lead to further pollen limitation. If, on the other hand, viable seeds are produced, this could lead to a hybrid. Gene flow between wild *E. angustifolia* and planted *E. pallida* could potentially have ecological consequences. Van Gaal et al. (1998), for example, looked at the consequences of crossing a wild type *E. purpurea* with a cultivated variety, and found higher reproductive output in the hybrids. This is not always the case, however, and thus understanding potential hybrids may help in assessing a threat level (Ruesink et al. 1995).

Research Question

1. Does the pollen of *Echinacea pallida* cause style shriveling in *Echinacea angustifolia*?
2. Does the pollen of *Echinacea angustifolia* cause style shriveling in *Echinacea pallida*?

Methods

In order to answer our research questions, I will use reciprocal pollen crosses between *E. pallida* and E. *angustifolia*.

Potential Further Questions

Styles may shrivel due to receiving compatible pollen. Is the *E. pallida* cytotype significant in whether these crosses are successful? A polyploid may contain more than two different S alleles, and thus a potentially lower chance of finding a compatible mate. Do these crosses lead to full or empty achenes? Full achenes would suggest that seeds were produced, and if that were the case, are those seeds viable? If so, traits of the hybrid may be of interest. Are the seeds fertile? What is the influence of the parental *E. pallida* cytotype on the hybrid viability? What is the offspring's cytotype?

Preferences

I would like to gain experience in analyzing data. I am not planning on working on this project with another transient member of Team Echinacea.

Plan B: The influence of aphids on pollen viability in *Echinacea angustifolia*

Background

Echinacea angustifolia is pollen limited (Wagenius 2004). Pollination failure can occur at one of three times: (1) before dispersal, (2) during dispersal, or (3) after dispersal. Examples of pre-dispersal failure include floral destruction or pollen being consumed. Dispersal failure can occur if a pollinator fails to get pollen to a compatible flower. Post-dispersal failure can occur a number of ways, including the receiving of incompatible-pollen, pollen clogging, allelopathy by other species' pollen, and receiving non-viable pollen (Wilcock and Neiland 2002). It has been observed that while *E. angustifolia* is pollen limited, the limitation is not due to a lack of pollinators (Wagenius and Lyon 2010). Other aspects of pollination failure must be investigated.

Pollen viability can vary due to a number of factors. Time after anther dehiscence, temperature, humidity, and species have all been known to influence pollen's viability. In many species, this viability can decrease quickly. Especially well known for this short viability are plants with trinucleate pollen, with the usual examples of plants in Poaceae and Asteraceae (Kearns and Inouye 1993).

Other factors influencing viability are also being examined. Herbivores may influence this trait. For example, in *Cucurbita texana* herbivore damage due to diabroticite beetles on the leaves was shown to decrease the quantity and quality of pollen (Quesada et al. 1995). The aphid *Aphis echinaceae* is a specialist of *E. angustifolia* (Ridley et al. unpublished). Work is needed to understand the effect of this aphid on plant fitness, including male fitness.

Research Question

1. Does the presence of aphids affect the pollen viability of *Echineacea angustifolia*?

Methods

Pollen viability test results can vary depending on the test (Haung et al. 2004). For this reason I will use two different methods:

1. Hauser and Morrison's (1964) nitro blue tetrazolium stain. This stain requires Sorensen's phosphate buffer (pH 7.4), Sodium succinate, Nitro-BT, and Sodium amytal. It also must be incubated at 37 C. Glass slides and other microscopy equipment will also be needed. F.A.A. is used as a fixative.

2. Cook and Stanley's (1960) triphenyl tetrazolium chloride stain. This stain requires 2,3,5-triphenyl tetrazolium chloride, as well as microscopy equipment.

Preferences

I would like to gain experience in analyzing data. This work would go alongside Katherine's aphid exclusion and addition experiments.

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