Plant Cultivation

Cultivation of Parasitic Flower Plants for Research and Teaching

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Approximately 1% of all flowering plant species are parasitic. Displaying extremes of specialization and development, parasitic plants are cherished botanical oddities. Apart from their appeal to botanists for their curious lifestyle, they have also been studied extensively, due to their economic impact or extremeness of adaptations. Aside from taxonomic studies, which have shown multiple independent evolutionary origins of parasitism[1], parasitic plants are generally classified depending upon their attachment points or by their degree of host dependence. The connection organ, called a haustorium, can attach to stems, as do the Mistletoes, or to roots, as do Beechdrops (*Epifagus*). Endophytic parasites, like *Rafflesia*, grow inside their hosts and only emerge to flower. *Rafflesia*, which exclusively parasitizes vines in the grape family, produces the largest flower of any angiosperm! It is also useful to divide parasites based upon their host dependence. One way is to describe the dependence on he host for carbon, or alternatively, on levels of parasite photosynthesis. Hemiparasites still photosynthesize, while holoparasites have lost photosynthesis and rely completely upon the host for carbon. Curiously, some holoparasites are thought to synthesize small amounts of chlorophyll. The role chlorophyll plays in a non-photosynthetic, obligate, holoparasite like *Phelipanche* is unclear[2].

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Questions like this drive scientific inquiry into parasitic plants, and the emerging answers have far reaching implications for plant biology. Parasitic plants, especially living examples that challenge standard botanical teachings of what parts and processes comprise a plant, are also extremely interesting to students of any age. This article will highlight cultivation methods for parasites from three plant families (Orobanchaceae, Convolvulaceae and Loranthaceae) that are grown and studied at Penn State, and in the labs of our collaborators. These plants represent a wide range of parasite types, including widely varying plant structures and growth rates. One of the primary hurdles in cultivation of parasitic plants is creating ideal conditions for the parasite to establish with a host that are also amenable to research goals.

Orobanchaceae

Contributed by
Loren Honaas and Claude dePamphilis

Consisting of 90 genera and ca. 1800 species[3], this family represents a special case in that it is the only parasitic plant family with species displaying the full range of parasitic abilities plus a free-living basal lineage, *Lindenbergia*, that is sister to the parasitic Orobanchaceae. This makes for an ideal comparative framework to understand the evolution of parasitism and the processes central to the parasitic lifestyle. Additionally, the parasitic Orobanchaceae are the most destructive parasitic weeds, causing $US billions of damage each year to agricultural crops in Africa and the Mediterranean[4]. Previous efforts to control parasitic Oroban-chaceae have been focused upon developing resistant hosts through mostly traditional breeding programs. These efforts have been met with limited success, so the search for new control strategies became part of the impetus to focus on the parasites. The National Science Foundation's Plant Genome Research Program funded the Parasitic Plant Genome Project (PPGP)[5] and continues to support parasitic plant research in the lab of Dr. Claude dePamphilis and collaborators, including John Yoder (University of California), James Westwood (Virginia Tech), and Michael Timko (University of Virginia).

One of the focal species of the PPGP is *Triphysaria versicolor* (yellowbeak owl's clover). This facultative, generalist parasitic plant has a long history of botanical interest and has been developed as an experimental model for parasitic plant research. It is one of four plants that are the focus in the current phase of the PPGP. The others are *Lindenbergia philippensis*, *Striga hermonthica* (an obligate hemiparasite), and *Phelipanche aegyptiaca* (an obligate holoparasite). An ongoing series of experiments is underway to explore host dependent growth patterns in *Triphysaria*. This work presents additional challenges of parasite-host co-culture since the experiments are designed to uncover parasite phenotypes that are correlated with exposure to different hosts across its broad host range (which spans flowering plants). At Penn State, *Triphysaria* is grown with *Solanum, Medicago, Arabidopsis, Oryza, Zea,* and *Juncus*.

The parasite seeds are collected from native grass stands near Napa, CA. Cultivation of *Triphysaria* begins with scarification using concentrated sulfuric acid, surface sterilization using concentrated bleach, stratification, and finally germination on sterile petri-plates with a minimal-nutrient, low-sugar medium. At the same time that hosts are transplanted, the *Triphysaria* seedlings are moved to pots (when the first true leaves begin to emerge). To facilitate establishment of the parasites, pots are frequently misted during the first week (Figure 1). The soil mixture is, by weight, mostly sand and the other media component is primarily peat. This nutrient poor, high drainage media is supplemented with slow release fertilizer to support healthy plant growth over the course of experiments that can last 8 weeks or more. With helpful advice from greenhouse manager Scott DiLoreto, an automated, multi-channel watering system was designed with parts from the hardware store. One version of the system was used to water 145 pots in 5 randomized blocks, each containing pots representing 7 treatments for a total of 16 independent channels (4 channels served the controls), and only cost $600 (Figure 1). The system was calibrated to deliver a small volume of water to each pot 2-3 times per day.

Figure 1.
Clockwise from top left; *Triphysaria* co-cultured with Tomato (*Solanum lycopersicum)* ~2 days after transplant; *Triphysaria* ~2 weeks after transplant; the automated watering system made with parts from local hardware store; and *Triphysaria* ~6 weeks after transplant with inflorescences.

Figure 2.
***Orobanche californica* growing on roots of host plant *Grindelia integrifolia*.**
Clockwise from top left: *Orobanche* plus *Grindelia* in 5" pot; removed from pot, showing three flowering *Orobanche* and smaller plants of various stages; close-up of small parasite seedlings attached to *Grindelia* roots.

This kept soil moisture high, supporting healthy *Triphysaria*, but drained sufficiently to prevent waterlogging of host roots.

The experiments are planned during spring and fall in central Pennsylvania to avoid excessive temperatures, and receive supplemental lighting as necessary. Diloreto has biological control methods at the ready and prefers to avoid spraying whenever possible. This conservative pest control approach allows tight control of experiments leading to cleaner data. These experiments have revealed host dependent phenotypes that are being explored in axenic growth conditions that favor experimental molecular approaches. It is hoped that understanding host-dependent phenotypes will shed light on host selection/preference mechanisms and parasitic plant-host plant communication.

Another Orobanchaceae species that has been cultivated at Penn State is *Orobanche californica*. This North American native holoparasite is grown on the perennial host *Grindelia integrifolia* (Figure 2) and has been used frequently in teaching demonstrations at Penn State. As a holoparasite that lacks photosynthetic activity, it has been routinely included in our examinations of parasite growth and next generation sequenc-

ing experiments. The transcriptome of *O. californica* was recently deeply sequenced to provide additional evidence for comparisons of several *Orobanche* species in the context of ongoing PPGP studies. Originally collected from the field by Dr. Alison Colwell (now a botanist with the National Park Service), these species have been co-cultivated continuously for more than 20 years. Native to Pacific Northwest coastal headlands, this pair grows well in a soil mix of 1 part metro 360: 1 part sand with supplemental light as needed. *Orobanche* plants will grow and flower almost year 'round under greenhouse conditions, spending the early part of their life underground and pure white, then emerging energetically from the soil as an inflorescence of purple flowers. Flowers will set seed without hand pollination, and will self-sow to maintain an active parasite-host culture for years. From time to time, the *Grindelia* plants need to be transferred to larger pots or propagated from cuttings with the addition of fresh *Orobanche* seeds to the soil mix.

Several other parasitic Orobanchaceae have also been grown successfully in our greenhouses, including *Schwalbea americana*, *Buchnera americana*, and multiple species of *Castilleja* and *Agalinis*. For all of these hemiparasites, slow-growing host plants worked well, especially native grasses like Blue Gramma (*Bouteloua gracilis*) that could be trimmed back/mowed/grazed to allow light to reach the young parasites. More importantly, all of those species require light for germination. The seedlings are so tiny that it's usually helpful to let them get a few sets of true leaves before starting the host or creating a 'host-free' area of the pot in which they can get established without being shaded out.

A long-term goal of studying parasitic Orobanchaceae is to leverage understanding of parasite biology in the fight against parasitic plants. Knowing more about the parasitic plant lifestyle will aid in the development of novel control strategies for the pernicious, weedy Orobanchaceae. The dePamphilis group publishes parasitic plant research regularly; check http://cwd.huck.psu.edu/ for more information about our current research efforts and publications.

Loranthaceae (Santalales)

Contributed by
Marcos Caraballo-Ortiz

This family is one of several in the order Santalales that contains species displaying the so-called mistletoe habit. It appears that within the order, multiple lineages of hemiparasitic stem parasites evolved the mistletoe habit independently[6]. In Loranthaceae, which consists of ~1000 species, most are mistletoes. Of particular interest in this family are *Dendropemon* mistletoes, which are restricted to the Caribbean Islands, and present a unique opportunity to understand plant evolution on insular ecosystems from the perspective of a parasite.

These hemiparasitic plants depend on woody hosts to obtain water and mineral nutrients. Although they are photosynthetic plants, Loranthaceae and other mistleoes are slow growers; they can take many months to fully establish and will only occupy a small space in the greenhouse for years. Because of their slow growth rates, these can be thought of as the "tortoises" of the parasite world. Seeds of mistletoes are short lived, and ger-

Figure 3. Clockwise from top left: *Dendropemon* mistletoe seed with the fruit flesh removed showing strands of sticky viscin; seed planted on *Citharexylum spinosum* (Verbenaceae); seedling producing first pair of leaves on *Thespesia populnea* (Malvaceae); seedling established on *Thespesia*; seedling developing epicortical roots on *Thespesia*; seedling arrested in growth at the cotyledon stage on *Tabebuia heterophylla* (Bignoniaceae).

mination is initiated as soon as the fruit is removed from the plant. Thus, mistletoe seeds are planted on branches of host trees shortly after being removed from the plant. To preserve freshness of fruits, they are collected while still attached to the infructescences (even better if collected with stems), placed in brown paper bags, and maintained in cool temperature (~4°C) for no more than three days. After three days, there is a high probability that the sticky substance surrounding the seeds (viscin) will degrade, decreasing the likelihood that seeds will anchor on host stems. The fruits are peeled to completely expose the seeds because the fruit flesh can interfere with seed germination and promote growth of pathogens (Figure 3). In the wild, the fruit flesh is removed and degraded in the digestive tract of birds before deposition on tree stems. Some mistletoe species have most of their viscin concentrated into a 'disk' at the top of the fruit that can be detached easily if fruits are peeled incorrectly or if the fruits are not ripe enough. After peeling, seeds are pinched between two fingers to remove some viscin for application directly on the host branch. It is recommended to leave the viscin drying for one or two days before exposure to water, and hosts should be watered in such a way to avoid dislodging seeds from host branches.

Although mistletoe germination is often very high, not all seeds germinate and sometimes seedlings will fail to make haustorial connections to host branches. In addition, mistletoes (especially tropical ones) are sensitive to overdrying by low humidity during germination, which can cause incomplete cotyledon emergence. Water quality can be an additional factor to take into consideration in greenhouses, as hard water can accumulate mineral deposits on young tissues. Last, selection of host trees is crucial for successful establishment of mistletoes, as not all mistletoes can be established on all tree species. Finding a suitable host will depend largely on the species of mistletoe to be planted. In the greenhouse we have planted seeds of tropical mistletoes from the Loranthaceae family (*Dendropemon* spp.) on a variety of hosts including known hosts for each species. Mistletoes established better and growth faster on their known hosts while seeds planted on hosts not known for the species either grow slowly, remain arrested in growth (at cotyledon stage) for an indefinite period of time, or die.

The work underway uses traditional taxonomy and modern molecular techniques to build high-resolution molecular phylogenies. This will clarify the taxonomy of the group and advance our understanding of the factors that might be involved in species diversification such as host choice, environmental conditions, and geography. The long-term goals include understanding how long-term interactions between parasites and hosts can promote speciation and host specialization in island ecosystems.

Figure 4.
Clockwise from top left: Cuscuta seed germination on filter paper after scarification; initial seedling attachment to host; mature vines in flower; newly transplanted seedlings.

Convolvulaceae

Contributed by Joel McNeal

Cuscuta is the only parasitic genus in this family and contains about 200 species. Also called dodder, this obligate stem parasite has a broad host range including many flowering plants, but not grasses. In contrast to the mistletoes, *Cuscuta* plants have the ability to grow at a surprisingly fast rate and die soon after seed production; *Cuscuta* represents the jackrabbit in our 'tortoise and hare' comparison of parasitic plant growth rates. Some species are mild to moderate agricultural pests, and they have recently made news because they seem to trade large amounts of RNA with their hosts[6]. This represents the first such observation and may provide insights into how parasitic plants communicate with their hosts. *Cuscuta* species have been grown at Penn State for systematics, chloroplast genome studies and volatile sensing studies and are currently grown at Kennesaw State University for studies on evolution of nuclear photosynthetic genes, haustorial development, and host specificity. Their fast life cycle, broad host range, and above-ground haustoria make them an excellent model for studying the evolution of parasites.

Cuscuta seeds can either be collected as soon as capsules are swollen or well after maturity. If they are still soft, the seed capsules can be left to dry in low humidity conditions until the seed coat has hardened. When stored at dry conditions either at room temperature or refrigerated, seeds can be viable for decades provided they aren't exposed to high heat; 40-year old seeds have even been successfully germinated from herbarium specimens that weren't placed in a heated dryer. Like their Morning Glory relatives, they have an extremely tough outer seed coat that must be scarified in order to allow water permeability and germination. The most effective means for achieving synchronized germination is to use concentrated sulfuric acid as the scarifying agent[8]. Porcelain Gooch Crucibles with a pore size small enough that the seeds won't fall through or become lodged in the pores are useful in transferring seeds between beakers to avoid contact with the acid. Thirty minutes to one hour in acid is usually sufficient to allow permeability for most *Cuscuta* species, and periodic agitation during that time can increase evenness of germination. The acid should be rinsed off thoroughly with water and the seeds briefly transferred to a weak bleach solution to sterilize the seed surface. After a final rinse, the seeds can be placed on wet filter paper in a petri dish and sealed.

Seeds will frequently swell within 24-48 hours and germinate shortly thereafter, although germination may sporadically occur over weeks or even months if the seeds were insufficiently scarified. A swollen, root-like hypocotyl emerges first, and the seedling gradually sheds the seed coat as the stem uncoils. Seedlings are best transplanted as soon as they have shed their seed coat. The hypocotyl doesn't absorb nutrients but should be kept wet and can be immersed in a small microcentrifuge tube filled with water as long as the yellow shoot sticks out from the tube (Figure 4). Most of the tube's opening can be covered with parafilm to prevent the water in the tube from evaporating until the seedling is established. The seedlings are very fragile to bruising or folding at this stage, and they usually will not survive if the stem is folded during transplanting. While almost any non-grass host can be used for mature plants, a host with a thin, vertical shoot should be used during initial seedling establishment, and the microcentrifuge tube can be placed in the soil next to the host (Figure 4). The host plants should be watered carefully to prevent damaging or dislodging the *Cuscuta* seedling until it has formed its first tight coil around the host stem. *Cuscuta* will not attach successfully if strictly grown under artificial light as the searching motions of the seedlings are coordinated by natural sunlight cues.

After a few days, the parasite stem will swell and commence rapid growth, after which it can be placed near other larger hosts. Each stem tip from the branching vines can grow inches per day, and most species will quickly cover an entire greenhouse bench if allowed to grow unabated. Any other greenhouse plants that you don't want parasitized should be kept well away from the growing *Cuscuta* vines, and the *Cuscuta* stems can be trimmed back liberally without killing the parasite. Growth will slow considerably once they commence flowering, and most species die after they have produced hundreds or thousands of seeds. New plants will have to be started every few months to maintain them in a greenhouse collection long term. The most common greenhouse pests on *Cuscuta* are aphids. Maintaining low greenhouse thrip levels at the time of planting is essential as they find *Cuscuta* seedlings particularly attractive and can kill the fragile seedlings with a single bite.

Conclusion

The cultivation of parasitic plants, while challenging, is a rewarding endeavor. Seeing the extreme adaptations firsthand can have a lasting effect on the observer and frequently generates enthusiasm among students of all ages. The few examples reported here represent years of careful experiments and observations that led to reliable methods for parasitic plant growth. Indeed challenges exist for many parasitic plants that have not been overcome, especially for the endophytic parasites, such that they have not been successfully cultivated. Pressing research questions and curiosity will continue to drive exploration of parasitic plant biology at all levels study. If you have questions or comments regarding how to grow parasitic plants, especially about the cultivation of parasites for teaching collections or outreach, we encourage the reader to reach out to the authors.

1. Barkman T. J., Lim S. H., Salleh K. M., Nais J. Mitochondrial DNA suggests at least 11 origins of parasitism in angiosperms and reveals genomic chimerism in parasitic plants. *BMC Evolutionary Biology.* **7** (2007)
2. Wickett N. J., Honaas L. A., Wafula E. K., Das M., Wu B., Landherr L., Timko M. P., Yoder J. I., Westwood J. H., dePamphilis C. W. Transcriptomes of the Parasitic Plant Family Orobanchaceae Reveal Surprising Conservation of Chlorophyll Synthesis. *Current biology.* **21**, 2098-2104 (2011)
3. Nickrent D. *The Parasitic Plant Connection*, http://www.parasiticplants.siu.edu/
4. Parker C. Parasitic Weeds: A World Challenge. *Weed Science.* **60**, 269-276 (2012)
5. *The Parasitic Plant Genome Project*, http://ppgp.huck.psu.edu/
6. Vidal-Russell R. & Nickrent D. L. The first mistletoes: Origins of aerial parasitism in Santalales. *Molecular Phylogenetics and Evolution.* **47**, 523-537 (2008).
7. Kim G., LeBlanc M. L., Wafula E. K., Depamphilis C. W. & Westwood J. H. Genomic-scale exchange of mRNA between a parasitic plant and its hosts. *Science.* **345**, 808-811 (2014)
8. Gaertner E. E. Studies of seed germination, seed identification, and host relationships in dodders, *Cuscuta* spp. *Memoirs of the Cornell University Agricultural Experiment Station*. 3–56, 294. (1950)

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