# A Practical Guide for Growing Dodder for Research

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### **INTRODUCTION**

Some plant parasites are themselves plants. These are the parasitic weeds, and they obtain water and nutrients from other plants rather than relying on their own roots and photosynthetic systems. Over 1% of plant species are parasitic, and parasitism in plants has evolved 12-13 times. The multiple evolutionary origins of parasitism account in part for the large diversity among parasite species in growth form, host preference, and reproductive strategy, but they all have a unique structure, termed the haustorium, which forms the physical and physiological bridge between the parasite and its host. The haustorium of parasitic plants shares is a multicellular organ that attaches to, penetrates, and forms vascular connections to a host plant. Unlike non-parasitic weeds, which merely compete with other plants for resources such as light, water, and nutrients, parasites tap directly into the host, removing resources and altering host physiology. Furthermore, they grow in close association with the host and can be extremely difficult to control, so parasitic plants are regarded as some of the most damaging and intractable weeds in the world.

An important characteristic in categorizing parasitic plants is the level of dependency on the host. <u>Facultative</u> parasites are opportunists that parasitize neighboring plants while retaining an ability to live independently. <u>Obligate</u> parasites, in contrast, have an absolute requirement for a host and often cannot develop beyond the seedling stage without forming an attachment to a host plant. Obligate parasites are further divided into <u>hemiparasites</u>, which are capable of some photosynthesis, and <u>holoparasites</u>, which lack photosynthetic capacity and must derive all their nutrients from the host. Another important concept for parasitic plants is <u>host specificity</u>. All parasites are specialized to some degree to use certain species as hosts, while other plant species will not support the parasite. Host ranges may be relatively narrow, consisting of only a few species, or may include species from several different families.

# DODDER

Dodders are vining parasites that attack stems and leaves of their hosts. These plants consist primarily of stem tissue that wraps around the host and forms haustoria at points of contact, with each connection fueling growth of news stems, each of which can parasitize additional host shoots. Through such prolific branching and formation of multiple connections, dodder may form dense mats of stems that simultaneously parasitize many host plants. Although most species of dodder have functional photosynthetic systems, they are generally yellow in color and rely primarily on hosts for all resources. Dodders can be very destructive, greatly reducing yields of parasitized crops, and can also be mildly toxic to livestock if consumed with infested forage. Dodders occur throughout the world and affect a wide range of herbaceous and woody dicotyledonous plants. They are a problem in forage legumes and horticultural crops, but do not survive well on grasses. Dodder seeds have a tough seed coat that is able to withstand passage through an animal gut, but otherwise have no special germination mechanism (in contrast to other classes of parasitic plants). They germinate in the spring along with other plant seedlings and produce a thread-like shoot that emerges from the soil and coils around nearby plants. This shoot may attempt to form haustorial connections with any vertical object, but will only succeed and grow after attachment to an acceptable host plant. Once established, the parasite will continue to spread and may form connections with a wide variety of plants.

# PROCEDURES

#### **1. PREPARING THE HOST**

Start growing host plants in advance of planting dodder seeds so that hosts are ready to support the parasite. The precise time of planting will depend on the specific host and the objectives of your experiment, but when you are starting dodder from seeds, it is best to have host plants that are also very young (seedling stage) or with thin stems to be inoculated.

- 1.1 Prepare pots or flats with potting medium (any soil or medium appropriate for growing the host plants will work).
- 1.2 Select host species to use. Some hosts are preferred, and this may depend on the species of dodder in questions. These recommendations are based on our experience with *Cuscuta pentagona* or *C. campestris*). Some host plants may be parasitized with more or less ease by the parasite, while some are non-hosts and will not serve as primary hosts, but may be parasitized by Cuscuta that are already growing on one or more other hosts. See Table 1.

Good hosts:			Non-hosts:
Solanaceae	Others		Poaceae
Tomato*	Alfalfa	Medicago	Sorghum
Pepper	Clover	Arabidopsis (bolting)	Millet
Tobacco	Bean	Beet	Wheat
Petunia	Carrot	Coleus	Fescue

**Table 1**. Potential host species to use in studying host preference in dodder.

\*Although our dodder comes from tomato fields, it often has trouble establishing on tomato hosts unless the tomato is a seedling. Tomato is a great secondary host.

#### 2. INOCULATION FROM SEEDS

*C. campestris* seedlings can be inoculated on hosts by either of two methods: planting the seeds near the host and waiting for "natural" attachment or pregerminating seeds on filter paper and moving them to be on or near host stems. In either case, the seeds must be released from dormancy by scarification of the seed coat. The best way to stimulate germination is by soaking in acid, to simulate passage through an animal digestive system, although some people use physical abrasion or cutting of the seed coat.

#### 2.1 Scarify C. campestris seeds for germination

- 2.1.1 Place seeds in a beaker with concentrated sulfuric acid for 20 60 min
- 2.1.2 Carefully decant acid from the seeds (or otherwise remove seeds from the acid).
- 2.1.3 Rinse dodder seeds in several (3-5) changes of tap water stirring constantly. A black layer will fall off from the seeds surface.
- 2.1.4 Proceed immediately to steps 2.2 or 2.3, or let seeds dry on a filter paper for short-term storage.
- 2.2 Dodder seed planting
  - 2.2.1 Plant the scarified dodder seeds 5-7 days before you want them to attach to hosts. Plant seeds by mixing them < 1 cm deep into the soil around the host plant with a small spatula. Dodder seedlings will begin emerging within a week of planting, and may continue to emerge sporadically for two or three more weeks.
  - 2.2.2 After dodder emergence, avoid growing plants under fluorescent lighting. Lights with a spectrum emphasizing blue or far red wavelengths will promote coiling, while fluorescent light inhibits coiling. Natural daylight also works well. We use lights shifted toward the blue spectrum to encourage attachments, often alone or in supplement to fluorescent lights. Some people put plants under red light when dodder has coiled as the red light promotes haustorial formation. Note that many dodder researchers have noticed seasonal effects: Dodder grows better and attaches more readily in spring and summer than in fall or winter (regardless of whether you are working in a greenhouse or growth chamber).

#### 2.3 Inoculation using seedlings

Note: Seedlings can be located directly on host stems. This method increases the coiling and attachment rate of dodder on the hosts. However, since seedlings are fragile, care is needed in manipulating seedlings to avoid damaging them.

- 2.3.1 Seed germination can be done *in vitro*. Once dodder seeds have been scarified, they are germinated on wet filter paper in a Petri dish at a temperature between 20-30°C. Seeds germinate after 3-5 days and can then be positioned on a host stem using an assisted method.
- 2.3.2 Seedlings may be fixed directly on the host stem. Carefully place a seedling on a young host stem, in parallel direction to the host stem, and then secure it with a narrow piece of tape positioned 1-2 cm below the dodder shoot tip. Seedlings should coil and make successful attachments within 4-7 days. Seedlings attached by this method are susceptible to desiccation, But enclosing host and parasites under high relative humidity conditions during the first days after inoculation improves success.

2.3.3 Alternatively, the seedling may be placed root-end-down in a small Eppendorf tube with water and the tube taped to the host stem with the shoot of the parasite protruding. This minimizes physical damage to seedlings and maintains moisture.

# PROPAGATION

The easiest method for propagating dodder is through stem cuttings. Once a parasite has attached to a host and shoots have started to grow, it will branch profusely and make additional attachments. To propagate, cut about a 6-10 cm section of dodder shoot, and gently tape it to the stem of another plant. This is best done using a very thin piece of scotch tape placed 1.5 cm below the tip of the dodder shoot/tendril. The dodder tip will coil around the host stem over the next 12 hours and then not develop haustoria over the next 4-5 days. Once the haustorial attachments are made, the dodder will resume growth.

# **SEED PRODUCTION**

*C. campestris* flowers are self-compatible, and seeds can be collected from plants that have flowered and dried out.