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Growth Assay for the Stem Parasitic Plants of the Genus *Cuscuta*

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[Abstract] *Cuscuta* spp. are widespread obligate holoparasitic plants with a broad host spectrum. Rootless *Cuscuta* penetrates host stems with so called haustoria to form a direct connection to the host vascular tissue (Dawson *et al.*, 1994; Lanini and Kogan, 2005; Kaiser *et al*., 2015). This connection allows a steady uptake of water, assimilates and essential nutrients from the host plant and therefore enables *Cuscuta* growth and proliferation. To quantify the parasites' ability to grow on potential host plants one can use the quantitative growth assay (Hegenauer *et al*., 2016) described herein, which exclusively utilizes fresh weight measurement as readout.
Keywords: *Cuscuta reflexa*, Dodder, Growth assay, Haustoria, Holoparasitic plant

[Background] In research fields of plant-pathogen resistance, either in basic research or in economic plant breeding, it is unavoidable to have an assay to quantify resistance against pathogen infection. To quantify the resistance/susceptibility of different plants against *Cuscuta* infections the simplest way is to measure the gain of biomass of *Cuscuta* growing on a plant of interest. This is a reliable method since *Cuscuta* is a holoparasite and its gain of biomass is completely depending on its ability to successfully infect another plant. Thus, unsuccessful infection of a plant leads to a decrease in biomass and subsequently the death of the parasite *Cuscuta*.

Materials and Reagents

1. Gloves and lab suit (*Cuscuta* sap causes stains on skin and clothes)
2. Mature *Cuscuta* (*e.g.*, *Cuscuta reflexa*; see Note 1 for cultivation)
3. Putative host plants

Equipment

1. Weighing machine/balance (mass range between 0.01-100 g)
2. Wooden planting rods (bamboo; diameter appropriate to the particular host plants stem diameter), available in gardening shops
3. Scissor to cut *Cuscuta* shoots

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Procedure

The whole experiment is performed under optimal conditions for the host plant. The optimal host plant conditions are provided by the seed supplier and can't be generalized.

1. Cut *Cuscuta* shoots (length ~15 cm) including the shoot tip from a mature plant and wind it around upright wooden sticks; be aware of winding direction (counter clockwise from bottom to top; Figure 1A) and integrity of the tip (see also Note 2). Keep the stick with the *Cuscuta* shoot in a vertical position for one day (*e.g.*, by sticking it into Styrofoam, soil or sand). The *Cuscuta* shoot should hold by itself on the planting rod without contact to anything else (Figure 1A).

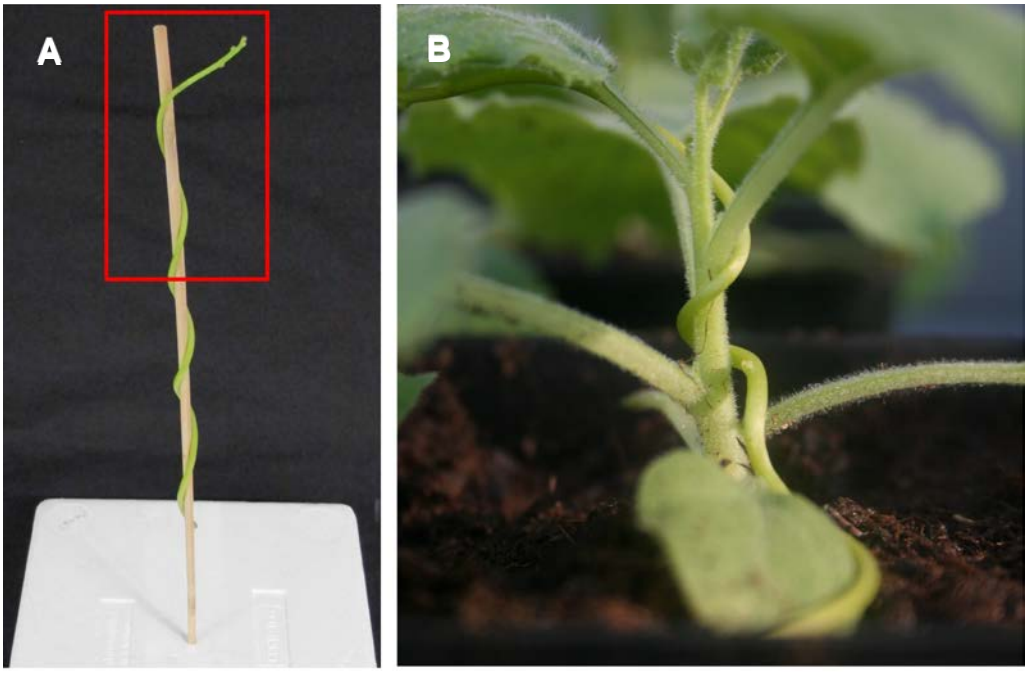

Figure 1. Starting the *Cuscuta* infection. A. *Cuscuta reflexa* shoot enwinding a wooden stick. Winding direction is counter clockwise from bottom to top. Red frame indicates the area where haustoria will most likely form. B. *Cuscuta reflexa* shoot wound around *N. benthamiana* plant after preconditioning around the wooden stick (A).

2. After one day (at the beginning of prehaustoria formation), when the shoot has been carefully uncoiled from the stick, weigh the shoots and transfer them to their host plants considering the winding direction (see also Note 3). The haustoria will form close to the shoot tip (Figure 1, red box) so make sure to wind this part around the host plant stems thoroughly. Remaining of the *Cuscuta* shoot will provide a source of nutrients and water until the haustoria connect with the host's vascular system. Therefore, an equal length (*e.g.*, 15 ± 1 cm) as well as an approximately same weight (*e.g.*, 0.5-0.7 g) of the parasite shoots is relevant for reproducible results.

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3. After transfer (Figure 1B, see also Note 4), let the *Cuscuta* spp. shoots grow for the same time period (14-21 days). After that time of growth, remove individual shoots from the host plant and measure the fresh weight immediately for each individual.

Data analysis

Cuscuta's ability and speed to accumulate biomass depends on the number of haustoria to acquire nutrients, and thus, on the time frame a single shoot needs to establish a successful haustorial connection to the host's vascular tissue. For this reason, the variance of the *Cuscuta* growth can be occasionally high. A big number (> 10) of replicates and multiple repeats are recommended. The outcome of this experiment is the gain of biomass (in g) for every single *Cuscuta* shoot for the distinct timeframe. So resistance against *Cuscuta* (or susceptibility for the infection) can be quantified and compared by its ability to gain biomass on two distinct sets of host plants. A correction factor is not necessary if there is no great variance in the initial weight, never the less if this is the case it could be helpful to show the result as mass change (ΔFw = final shoot weight - initial shoot weight) during the time frame. This depends on the particular experiments experimenters will perform to answer their research questions.
The results can be presented as the mean of the *Cuscuta* shoot biomass 21 dpi (days post infection) of n replicates with standard deviation comparing two types of hostplants.
For the reduction of outlier effects, ranked data analysis and nonparametric tests like Mann-Whitney *U*-test can be used.
See also Note 5.

Notes

1. Cultivation of *Cuscuta*: *Cuscuta* can be cultivated on many plants. In our Lab we use Coleus (*Solenostemon scutellarioides*) because it's a robust plant and its red color gives a good contrast to spot *Cuscuta* shoots. You can easily cultivate *Cuscuta* when you place 6 to 10 host plants in close proximity so that *Cuscuta* can overgrow all of them. It's important to concern that after *Cuscuta* overgrows the host plant it cannot be potted anymore, so make sure to provide enough nutrients for the host plant. *Cuscuta* should be cultivated under long day conditions with 17 h daylight. *Cuscuta* seeds and instructions how to start the culture can be requested in University botanical gardens or other research groups.
2. The pre winding of *Cuscuta* around a wooden planting rod facilitates the later infection of the host plant due to an efficient prehaustoria formation, a clearly visible swelling of the shoot at the shoot-stick contact sites.
3. It is important not to harm the *Cuscuta* shoot during the initial weight measurement and transfer to the host plant.

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4. In the first days of infection the *Cuscuta* shoot may search in the surrounding area of the host plant for other hosts despite infecting the plant it is sitting on. Therefore, the experimenter has to softly redirect the shoot back to its host.
5. Blinded set up of the experiment: Comparison of *e.g.*, *N. benthamiana* wild type plants with resistant transgenic CuRe1-expressing plants was performed as blind experiment in Hegenauer *et al.* (2016). One person randomly assigned numbers to each tested plant and a second person performed the infection with *Cuscuta reflexa* shoots.
6. A successful infection of Coleus by *Cuscuta reflexa* is shown in Figure 2. At the side of haustorial infection *Cuscuta* is thickened (red frame). After successful infection *Cuscuta* starts growing and branching (blue arrow).

Figure 2. Coleus successfully infected by *Cuscuta reflexa*. Red frame: sides of haustorial development. Blue arrow: branching of *Cuscuta.*

Acknowledgments

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