



Repeating Crumley 3: The Setup

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DATE RECEIVED:

June 10, 2015

DOI:

10.15200/winn.142722.20079

ARCHIVED:

March 24, 2015

CITATION:

Anthony Salvagno, Repeating Crumley 3: The Setup, *The Winnower* 2:e142722.20079, 2015, DOI:

[10.15200/winn.142722.20079](https://doi.org/10.15200/winn.142722.20079)

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It's a new day and that means a new Crumley Trial. This is the 3rd try and hopefully one of the last trials (if everything goes smoothly). As always, I'm looking to repeat the results Crumley got back in 1960 growing tobacco seeds in D2O. Only I'm doing it better (no disrespect to Crumley, but there were some flaws in the original setup).

After getting the results of the water evaporation experiment I decided to go with the DOW Corning Vacuum Grease to seal the chambers. The product claims it has low reactivity with water and provides a great seal (according to my results). Since I want as little interaction with water as possible this seems like a viable option. With that said, let's move on to the setup.

I prepared 8 samples for this trial using a combination of DI water, DDW, and D2O. I'm using [Cuban Havana 2000](#) seeds from the Tobacco Seed Co (because the idea of growing Cuban cigar seeds in the lab sounds awesome!).

1. I counted 30 seeds per sample and placed them on weigh paper for addition to the samples after the water step (see below). Using weigh paper helped considerably when dealing with the static attraction the seeds tend to have.
2. 1 set of seeds (unknown amount) was placed in 8mL of DI water and left to presoak for 30 min. The water from this sample would be used as a control to monitor fungal and mold growth.
3. I placed the seeds aside and prepared the water for the samples. The 8 samples include: (1) di water (no seeds), (2) di water, (3) 33% d2o in di water (2mL d2o, 4mL di water), (4) 66% d2o in di water, (5) 99.9% pure d2o, (6) 33% d2o in ddw, (7) 66% d2o in ddw, and (8) 99.9% pure ddw.
4. 6mL of each water type was added to an analyslide (see Experiment Product page at top). The presorted seeds were then added to the water. The analyslides were then closed and sealed with Dow Corning Vacuum Grease.
5. For the control sample, seeds were removed before closing and sealing the chamber. Sealing the chambers is quite a task that I have turned into an art. When I put the analyslide cover on I put it on loosely at first. Then I tilt it vertically so the air bubble moves toward the slight opening at the top. I begin to press around the edges with my thumbs until it is closed completely. Finally I spread vacuum grease around the rim (at first with the no cotton end of a long qtip, but then I used some plastic innoculating loops we had because it was thinner) for the final seal.

I will provide a post on the sealing process later today or tomorrow depending on who can show up to take pictures of me doing this.