Burk, A.G. and A.M. Srb. Perithecial submersion: a method for detecting the effect of compounds on ascus development.

In an attempt to study the effect of antimitotic susbtances on developing asci, the ability of perithecia to survive submersion was examined. This was necessitated by the observation that transport of many, if not all, molecules from the mycelium to the developing perithecia ceases approximately 4-5 days after fertilization (Nasrallah and Srb, unpublished). Three lines of evidence suggest that certain molecules can enter the perithecium via submersion: 1) submersion of perithecia in ^{3}H-leucine results in incorporation of the label into perithecial protein (Nasrallah and Srb, unpublished); 2) perithecia grown on biotin-deficient media normally produce many indurated asci, but if such perithecia are submerged at an early stage in a biotin solution, only normal spores are produced. 3) When perithecia are submerged in a solution containing methylene blue, the dye enters the perithecial cavity within the first hour of submersion. Presumably, sub-

mersion of perithecia results in uptake via the ostiole.

The following is a description of our current procedure. Perithecia are collected from crosses made by spreading a conidial suspension of both parental strains on Westergaard's medium in petri plater. After the onset of ascus formation, but before the beginning of arcorpore formation, perithecia me collected with the aid of fine forceps and transferred onto 4% agar. After removing any excess Westergaard's agar medium adhering, a flattened inoculating needle is used to transfer the perithecio to 10 x 75mm test tubes filled with 1.5ml rubmerrion medium. Usually 15 perithecio are transferred to each of two tube,. The perithecia in one tube are left submerged and the peritheciol contents checked at appropriate intervals to determine whether further development, as evidenced by the formation of ascospores, accurs during rubmerrion. The perithecia in the second tube are submerged for a specific length of time (6hrs. works well), then poured into a filter-lined funnel, washed with about 30ml distilled water, and transferred to a slant of 2% purified agar. Perithecial contents are checked at intervals (usually 1, 2, and 3 days after transfer) to determine whether arcorpore formation proceeds normally.

The following parameters hove been tested for their effect on peritheciol development. a) Preliminary handling of perithecio -- when perithecio that have been "cleaned" by quickly removing excess agar and by washing in distilled water both before and after submersion me compared to perithecia not cleaned, no differences in arcorpore production ore seen. Thus, if care is token to ovoid dessication, the perithecia can be handled without ill effect. b) Perithecial stage -- in general, the older the perithecium, the better it survives submersion. When 3-4 day old material is submerged and subsequently transferred to agar, a few mature and form spores, but the majority deteriorate before forming asci. Most 4-5 day old perithecia survive rubmerrion and form ripe spores, but the frequency of ascus abortion is often higher than in unsubmerged prithecia. Perithecio that already contain asci, but no spores, usually survive submersion well, c) Submersion medium -- in most experiments involving several submersion media, distilled water was used as the "control"; and thus as a standard of comparison for evaluating the other media tested.

Following is a list of submersion media tested. In each core, peritheciol contents were examined qualitatively to determine the presence or absence of detrimental effects, such as increased ascus abortion, variation in spore size or shape, or increased frequency of 5-spored asci. -- Best, subsequent perithecial development: 5 units avidin/1 0.5% NaCl, Squibb mineral oil; Good: 0.05% NaCl, 50 units avidin/1 0.05% NaCl; Average: distilled water, liquid Westergaard's medium containing 0%. 2%, or 4% sucrose, liquid Westergaard's medium diluted 1:1 with distilled water, 5mM caffeine, 10 mM caffeine; Poor: liquid Westergaard's medium containing 8% sucrose, 8% sucrose solution, 0.05 M colchicine; Toxic: 0.1 M colcicine, 0. 1 M phosphate buffer (pH 6.7), 0.3 M acetate buffer (pH 5.2).

Requirements for completion of perithecial development during continuous submersion ore stricter than those for survival after temporary submersion. -- Good, comparable to an unsubmerged culture: 5 units avid/l 0.05% NaCl, Squibb mineral oil, 0.05% NaCl; Average: distilled water, 50 units avidin/1 0.05% NaCl; Poor, only a few spores form or that spores or asci ore abnormal: liquid Westergaard's medium containing no sucrose, 8% sucrose solution, 5mM caffeine; little or no further development; liquid Westergaard's medium containing 2%, 4%. or 8% sucrose, liquid Westergaard's medium (2% sucrose) diluted 1:1 with distilled water, 10 mM caffeine.

Several interesting facts emerge. Although Westergaard's medium is a widely used crossing medium, continuous submersion of developing prithecia in liquid Westergaard's medium inhibits further development, even when 6-day-old prithecia, which already contain young spores, ore submerged. Sucrose in the submersion medium also seems detrimental to further development. Also, once perithecia start forming asci, they seem to be self-contained, requiring no obvious source of nutrients (i.e., they will develop in distilled water) and little or no external oxygen (good development in mineral oil). Perithecia that have been submerged in distilled water for up to 7 days -- with no further development during rubmerrion -- will, upon transfer to agar, resume development and form normal spores. There doer, however, seem to be a correlation between the length of submersion before transfer and the amount of ascus abortion. A six hour rubmerrion seems to provide adequate uptake of the compound of interest and doer not usually result in increased ascus abortion.

There is also a relationship between the age of the perithecium and the length of submersion tolerated. For example, perithecio which contain only sterile hyphae and croziers will tolerate a six hour submersion in liquid Westergaard's medium quite well; but, if submerged for 24 hours, approximately 80% of the perithecia degenerate. Older perithecia, which already contain asci, will survive quite well after a 24-hour submersion in the same solution.

No differences in development were detected between perithecia transferred to purified agar and those transferred to agar containing Westergaard's medium. The advantage of purified agar is that subsequent hyphal growth and de nova peritheciol formation are kept at a minimum.

Submersion may be a useful procedure for a variety of studier. It can be used as a means for effecting perithecial uptake of nutrients, inhibitors, etc., and would be especially suited for compounds that are too unstable to be added directly to a crossing medium, or, conversely, for compounds that inhibit crossing per se. Results from continuous submersion may provide insight into the nutrients, etc. necessary for in vitro development of isolated asci. (This research was supported by a Predoctoral Training Grant, TI GM-01035, from the Notional Institute of General Medico, Sciences, USPHS, and by Grant GM-12953 (to A.M. Srb) from the Notional Institute of General Medical Sciences, USPHS.) - - - Section of Botany, Genetics and Development, Cornell University, Ithaca, NY 14853.