(Pre)Proposal for Funding by The American Chestnut Foundation

Title: Nutrient media for determination of sexual compatibility of *Cryphonectria parasitica* isolates.

Principal Investigator: Steven Jakobi, Ph.D., Assoc. Professor, Alfred State College (ASC), Alfred, NY 14802

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Description: The currently most widely used method for determination of sexual compatibility between two isolates of *Cryphonectria parasitica (C.p.)* involves co-inoculation of the strains into dormant American chestnut stems. The method is time-consuming (requiring an average 8-10 weeks for observable perithecial necks of the fungus in chestnut bark) and needs a ready supply of suitably-sized dormant stem pieces. Preliminary screening of 10 nutrient formulations has indicated that two solid media may be suitable for the production of perithecia in as little as 3-4 weeks under routine laboratory conditions. One of these, PDA amended with chestnut bark extract, still relies on availability of bark material, but appears to be at least partly successful in eliciting rapid production of perithecia with ascospores. The other formulation has had more limited success but most likely can be improved. Although initial research was supported by an ASC faculty development grant, refinement of the procedures requires additional supplies and time investment. Other planned media candidates also may prove more useful than the stem method in a more rapid and reliable production of sexual spores.

Timeline: On-going trials with completion in 1 year.

Budget: $ 1,000 for media ingredients and disposable Petri plates. Equipment and facilities are in place; unpaid undergraduate student help is in the form of credits earned.

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Project Description:

Since the accidental introduction of the chestnut blight fungus into North America about 110 years ago, and the subsequent spread of the pathogen throughout the natural range of the American chestnut, genetic diversity has apparently increased in *Cryphonectria parasitica* (Milgroom and Cortesi, 1999). The flow of genetic information between/among different populations of the pathogen depends on two factors: vegetative compatibility (VC), which determines whether hyphae of two different strains can fuse; and sexual compatibility, in which opposite mating types produce sexual spores (ascospores). Since sexual reproduction results in significant potential genetic recombination, it is often useful to determine the distribution of both VC types and mating types in a particular population of the pathogen. A complicating factor in determining these characteristics in a given canker, or a given tree, or a given locale, is that vegetatively incompatible strains may still be sexually compatible (Kuhlman and Bhattacharyya, 1984; Marra and Milgroom, 2001).

The currently most widely used technique for the determination of sexual compatibility between two isolates of *C. parasitica* involves co-inoculation of the strains into dormant American chestnut stems (Anagnostakis, 1979; Willey, 1980). This method may not work if the two strains are vegetatively incompatible. To overcome this problem, asexual spores (conidia) are first obtained by growing the mycelium on nutrient plates. The conidia are then used to spermatize the asexual reproductive structures (pycnidia) of the pathogen growing on chestnut stems. The technique is often time-consuming, requiring 8-10 weeks for development of observable perithecial necks in chestnut bark (M. Double, personal communication), and needs a ready supply of suitably-sized dormant stem pieces. While such material may be relatively easily obtained in the eastern U.S., researchers in many parts of the world do not have easy access to dormant stems.

The development of an artificial medium that would support the production of ascospores in a timely manner has been an elusive goal (M. Double, personal communication; M. Marshall, personal communication). Preliminary work at Alfred State College in 2012 utilized 10 different solid media to determine their suitability for production of perithecia that contain viable ascospores. Isolates of *C. parasitica* were obtained from the culture collection of West Virginia University, and included the Mating Type 1 strains "EP-146" (a brown-pigmented isolate found in West Virginia) and "Schomberg" (from Wisconsin); and the Mating Type 2 strains "EP-155" (originally discovered in Connecticut), "Bockenhauer" (from Wisconsin), and an isolate designated as "6-7-1" (from West Virginia).

Two solid media formulations appeared to be at least partially successful in production of perithecia in as little as 3 weeks after spermatization. One of these, potato dextrose agar (PDA) amended with chestnut bark extract, still relied on availability of chestnut material, but 7 of 12 crosses had observable perithecia and viable ascospores 15-28 days after spermatization. The other media, designated as Leonian agar (Tuitte, 1969) produced less consistent results. The initial crosses between the sexually compatible isolates EP 155 and EP 146 resulted in sparse perithecial production in 3 of 5 plates, and no ascospores in the EP 156 x Bockenhauer cross. A second, larger trial resulted in 2 of 6 EP155 x EP 146 plates exhibiting perithecial necks and ascospores, but none of the other crosses -- representing 20 additional mating pairs in various combinations -- was successful. A review of the protocols revealed slight differences in methodology between the first and second set of trials in the leonian media experiment. These differences can be the starting point for further trials and refinement of techniques.

The development of a simple, time-saving technique for the laboratory media-based identification of mating types of unknown isolates would greatly facilitate studies of the genetic make-up and structure of populations of *C. parasitica.* Toward this goal, I am respectfully requesting $ 1,000 from the Foundation. The funds would be used for the purchase of disposable sterile Petri plates ($ 252 per 500 units), nutrient agar ($ 198.50 per 500 gm), and other media (e.g. PDA @ $ 174.10 per 500 gm). No salaries would be funded from this grant, as unpaid student help is readily available in exchange for college credits in research techniques. Thank you for considering my request.

Literature cited:

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Marra, R.E. and M.G. Milgroom. 2001. The mating system of the fungus *Cryphonectria parasitica*: selfing and self incompatibility. Heredity 86:134-143.

Milgroom, M.G. and P. Cortese. 1999. Analysis of the population structure of the chestnut blight fungus based on vegetative incompatibility. Proc. Natl. Acad. Sci. 96:10518-10523.

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