Selecting Superior Turfgrasses Through A Tissue Culture System

by Dr. M.A.L. Smith
University of Illinois

Kentucky bluegrass is attacked each year by two serious and similar diseases — Summer Patch and Necrotic Ring Spot. Although two separate fungal pathogens cause these diseases, the fungi often act in concert, and combined they create a complicated disease interaction which is potentially even more destructive: the misnamed "Fusarium Blight Syndrome". This poorly understood complex has defied research studies, and as a result has actually been blamed on **Fusarium**, the wrong casual organism.

Not only do the interactions between the two organisms make the problem very difficult to study (and consequently, very difficult to **SOLVE**), but the scientist has no control over this disease for field testing. Any variation in soil type, drainage, or other environmental factor will change the expected expression of disease symptoms, AND the disease will not develop quickly enough after artificial inoculation to allow selection of resistant grasses.

These complications impede the work of any breeder trying to find superior selections without the disease problems, **or** trying to formulate effective control measures. In addition, a population of soil-borne bacteria (Pseudomonads) acts as an antagonist to the fungi. The bacteria are triggered to multiply and build up their population by some signal which comes directly from the grass + pathogen interaction. After a period of time, the bacteria can build to a sufficient population to effectively suppress the pathogenesis of the fungi, and give real protection to turfgrass from disease. We can't afford to wait for this biological control to work on high maintenance golf greens or in a landscape, but it would be a benefit to SELECT for bluegrass genotypes which are resistant to the pathogen **and** supportive of bacterial colonization. A selection of bluegrass may seem "resistant" to disease attack because it somehow is able to ward off the fungi, or it may show no symptoms because it is very receptive instead to the bacteria, and **they** are preventing any damage. Or maybe both are operative in a "resistant" grass — some genetic defense against fungi and receptivity to bacteria that colonize the rhizosphere.

The complexity of the disease problem, and the slow progress possible with field experiments warrants a simplified, alternative approach. What we would ideally like to do (in order to make some real progress) is to isolate the bluegrass in a defined, controlled environment, without the complications of a soil rhizosphere, or competing microorganisms. Then we could with precision introduce a specific pathogen (or bacterial colonist) to the plant, and observe the reason for protection, or the plant attributes which influence disease susceptibility, without interference. That is exactly what we **can** do by developing a microculture system for bluegrass. We can provide an ideal analog of the field situation by growing grass plants in an isolated environment on a medium we have defined and standardized, so that many of the complicating variables which have precluded research progress are eliminated. Now when we "add back" a specific organism of our choice, we can easily study the way the disease attacks or the manner in which the bacteria are able to offer some protection. If a grass shows some resistance to the disease, we can tell exactly why, and work in a directed

manner to breed bluegrass to fortify the mode of resistance. Remember, we are really looking for two separate traits (or a combination of these) which can contribute to a superior bluegrass line - ability to resist fungal attack because of some unique plant character controlled by its genotype, or a bluegrass that is receptive to rapid buildup of bacteria in the rhizosphere, and is then indirectly afforded some protection.

Our objectives in this present study are the following: 1. to establish a bluegrass microculture system using differentiated tissues (whole grass plants, or whole organs like blades [phytomers] or roots) in culture AND undifferentiated (callus) cultures. The microorganissms of interest in this artificial environment as well. 2. to correlate the level of field susceptibility to Summer Patch with a disease reaction observed **in vitro**. 3. to use this microculture analogy of a field disease to SCREEN and SELECT for resistant bluegrasses, without resorting to the slow, tedious, and usually inconclusive traditional methods of field evaluation. 4. to generate someclonal variants from the microcultures, to serve as another source of potential breeding material. Whether we are selecting for some natural resistance in grasses while they are in the microculture form, or actually creating new and potentially superior selections **through** the microculture process itself, our objective is get a grass plant that has demonstrated superior and desirable traits in culture, and acclimate it back to the greenhouse and eventually the field, so that we have produced a new selection that is useful to whole plant breeders. All of these evaluations and selections can take place **much** more rapidly than any similar tests in the field.

We now have completed a standardized, workable microculture system for bluegrass in all three of the culture forms mentioned. We can with confidence generate uniform tissue rapidly for screening and testing in pathogen or bacterial interaction assays, select or induce superior grass genotypes in culture, and regenerate whole field-ready plants which are then available to a breeder for further work. We have also begun testing the pathogen (isolated from all other organisms) in the culture environment, making sure that it is able to grow normally **in vitro**. And we've been fortunate to make use of a whole-plant growth chamber assay for disease susceptibility. In this ''cone-assay'', the selections made at the microculture level can be introduced into the greenhouse/growth chamber environment and very rapidly (28 day turnover) measured in terms of resistance; the disease can be produced consistently. We can't do selections or variant breeding in the growth chamber assay, but it provides a fairly rapid, specific check to verify that the resistance seen at the micro-level holds up in a natural environment. Remember, there is no such check available in the field, where the disease can't be controlled to do effective testing.

We are currently in final stages of preparing multiple uniform microcultures of bluegrass varieties with known levels of resistance and susceptibility to Summer Patch disease - specifically 'Adelphi' which has demonstrated some field resistance to Summer Patch and the combined problem caused by both the Summer Patch and Necrotic Ring Spot fungus, and 'Fylking' which is quite susceptible to damage. Their differential reactions to the fungi have been verified using the growth chamber assay mentioned earlier. Now, we are moving ahead to test both the causal fungus responsible for Summer Patch, and the grasses in all of the three microculture forms. Once we can determine the kind of response to be expected of a ''resistant'' grass in culture, we can use the rapid assay to select, isolate, and produce whole plants of immediate use in landscape situations.

Parallel experiments with wheat, attacked by the same or closely related fungus, have helped to verify the kinds of responses seen with bluegrass. So the microculture system is not only a feasible method, but perhaps the only way to make substantial progress towards control of a severe and challenging turfgrass disease.