FISHER, G.A., and J.S. GERIK, Holly Sugar Co., Plant Pathology Laboratory, Tracy CA 95378. A PCR-Based Method for Differentiation of Rhizoctonia solani Anastomosis Groups.

The fungus Rhizoctonia solani is one of the most widely distributed and important pathogens of sugarbeets. R. solani is a form species of morphologically similar fungi which differ greatly in their ecological attributes. The species is subdivided into Anastomosis Groups (AGs) based on the ability of isolates to fuse with one another cytoplasmically. Most researchers recognize eleven AGs within R.solani, some of which are further subdivided based on physiological differences. Only two AGs are known to cause disease in sugarbeets. These two AGs differ somewhat in the type of disease they typically induce. AG 4 usually causes "damping off" of seedlings, while AG 2-2 has the ability to cause rot of mature beets. Knowing which AG a particular isolate belongs to is thus crucial for predicting the potential for disease.

Polymerase Chain Reaction (PCR) amplification of fungal DNA sequences is a powerful tool for the taxonomic analysis of fungi. Recently, primers which amplify the Internal Transcribed Spacer (ITS) sequence of rDNA from many diverse organisms have been developed by White *et al.*(1990). This noncoding sequence is not highly conserved, thus making it a useful place to look for differences between organisms.

Fungal isolates were grown in liquid media for three or more days, then ground in liquid nitrogen and the DNA purified by organic extraction and isopropanol precipitation. Amplification of the ITS region using primers ITS 1 and ITS 4 yielded a product of approximately 750 bp. Twenty-five cycles was found to be adequate. When the PCR products were digested with restriction endonucleases , the Restriction Fragment Length Polymorphisms (RFLPs) generated showed clear differences between isolates of different AGs. So far, eight endonucleases have been studied, and four of these produced unique RFLPs for at least one of the beet-pathogenic AGs. Alu I and Mbo I produced unique RFLPs for AG 4. Hae III and Hpa II yielded RFLPs unique to AG 2-2. Several other enzymes gave unique RFLPs for other AGs, not pathogenic to beets. No one enzyme has been found which will allow differentiation of beet pathogenic AGs with a single digest, but since enough DNA can be produced in one PCR to run several digests this has not been problematic. This technique compares favorably with the traditional microscopy method for determination of AGs in terms of speed, and is much less subjective.