Diplodia shoot blight disease can cause significant damage on coniferous trees and be particularly injurious to cones, which reduces the amount of seed production and germination. We investigated the disease severity and genetic variation of *Diplodia pinea* in one *Pinus nigra* and two *P. sylvestris* seed orchards. Disease surveys were carried out in İzmit (Marmara region, Turkey) in May 2012. Symptomatic shoots and cones were examined for the presence of pycnidia. Cultural and morphological characteristics of the isolates were studied using cultures grown on potato dextrose agar (PDA). Based on morphological characteristics and results using species specific primers, the pycnidia on shoots and cones were identified as *D. pinea*. In addition, Random Amplified Microsatellite Sequence (RAMS) analyses indicated that there was a single genet of *D. pinea* which caused the disease in the seed orchards. All of the 60 sampled trees were found to be infected by the fungus. There were differences in disease severity among the stands.