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MOLECULAR DETECTION, IDENTIFICATION AND QUANTIFICATION OF PARATRICHODORUS ALLIUS FROM NEMATODE INDIVIDUALS, COMMUNITIES AND SOIL DNA. **Huang, Danqiong [1] , G.P. Yan [1] , A. Plaisance [1] , N.C. Gudmestad [1] , J. Whitworth[2] , K. Frost [3] , C.R. Brown [4] , S.L. Hafez [5] , Z.A. Handoo [6] , and A.M. Skantar [6] .**
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Paratrichodorus allius is one of the most prevalent species of stubby root nematodes distributed in the United States. It is the vector of Tobacco rattle virus, cause of corky ringspot disease. To manage or predict the occurrence of this economically significant nematode species, it is important to have a rapid, sensitive and reliable method for detecting and identifying this nematode under a wide range of conditions. The traditional morphological identification of stubby root nematode species is time-consuming and requires an experienced taxonomist due to similar morphometrics among closely related species. The goal of this research was to develop molecular protocols to detect, identify, and quantify this nematode species using DNA extracted from different materials, such as nematode individuals, nematode communities, or soil samples. In 2015 and 2016, nematodes were extracted from soil using the sieving, decanting and sugar centrifugal flotation methods, and stubby root nematodes were found in 109 soil samples from North Dakota, Minnesota, Idaho, Oregon, and Washington. Morphometric measurements identified the species as *P. allius* using nematode populations isolated from four fields (three from ND and one from MN). Meanwhile, genome sequencing, including regions of 18S rRNA, D2-D3 of 28S rRNA, and internal transcribed spacer (ITS) rDNA, confirmed the species identity in these four fields and further identified *P. allius* in 24 other fields. To avoid the cost of sequencing services, conventional species-specific PCR was developed. In addition, to be able to estimate nematode population densities, quantitative real-time PCR (qPCR) assays (TaqMan probe and SYBR green) were also developed. Primers/probe were designed from ITS1 rDNA of *P. allius*. Specificity of the primers and probe was evaluated by in silico analysis and confirmed by experimental qPCR tests with specific amplification using DNA of target species and nontarget nematode species. The conventional PCR and SYBR green qPCR detected and identified *P. allius* in DNA extracts of stubby root nematode individuals isolated from 35 soil samples and of nematode communities with mixed populations of nematodes isolated from 11 soil samples. In the qPCR assays for *P. allius* quantification, standard curves were generated using a serial dilution of soil DNA extracts from autoclaved soil harboring 10 *P. allius* individuals. The qPCR assay accurately quantified *P. allius* densities from DNA extracts of artificially infested soil, revealed by the correlation (r) of greater than 0.93 between the numbers of target nematodes quantified by two qPCR assays and added to the autoclaved soil. Using 17 natural field soil samples, the SYBR green qPCR assay (r = 0.93) performed better than the TaqMan probe qPCR assay (r = 0.89), indicated by a higher correlation coefficient between the qPCR quantified numbers and microscopically estimated numbers. Moreover, the numbers of

target nematodes estimated by the SYBR Green qPCR assay were closer to the actual numbers estimated by microscopic methods. Results of this study suggest it is feasible to use molecular diagnostic procedures for *P. allius* detection, identification, and quantification.