The potato cyst nematode, *G. pallida*, and the beet cyst nematode, *H. schachtii*, are major nematode pests in potato and sugar beet production, respectively. Their impact on the crop depends largely on pre-plant nematode density. Due to their damaging potential, potato cyst nematodes (*G. pallida* and *G. rostochiensis*) are subject of quarantine regulations in many countries; also *H. schachtii* is on the quarantine list of several non-European countries. Cyst forming nematodes are frequently present in mixed populations. Precise identification and knowledge about the number of nematodes in field soil are necessary to develop effective integrated pest control.

**SYBR green I fluorescent dye.** Advantages: (1) easy to use, (2) no sequence specificity, (3) detects any PCR product, (4) low cost, and (5) binds to any double-stranded DNA. Disadvantage: Dye binds also to any non-specific product including primer dimers. Melting curve analysis can overcome this problem.

**Melting curve analysis.** Non-specific PCR products tend to melt at a much lower temperature than the longer specific products. The length of amplicons can be used to differentiate amplification products separated by less than 2°C in Tm. The presence of only a single peak with melting temperatures of 84.6 ± 0.1°C and 84.1 ± 0.1°C was acquired for *H. schachtii* and *G. pallida*, respectively (Fig. 2).

**Amplification.** rDNA of *G. pallida* species was amplified with the species specific primers described by Bulman & Marshall (1997); rDNA of *H. schachtii* was amplified with primers slightly modified from Amiri et al. (2002). PCR performed with the specific primer combinations produced for all studied *G. pallida* or *H. schachtii* populations only the single expected fragment of about 115 bp (Fig. 3A).

**Validation test and detection limit.** DNA extracted from different precise numbers of juveniles was amplified by Real Time PCR. The numbers of *G. pallida* second stage juveniles present in the sample and the expected numbers detected by real-time PCR matched highly; the match was less perfect for *H. schachtii* (Fig. 4). The technique enables us to detect a single second stage juvenile of *G. pallida* or *H. schachtii* in a sample.

**Haplotype hypothesis.** The variation in detection and quantification of these two species might be due to differences in amount of the target rDNA. The specific primer designed for *H. schachtii* amplifies only one of the ITS-rDNA haplotypes of the species, whereas the primers for *G. pallida* amplify all haplotypes. Although we concluded that the haplotype amplified by the *H. schachtii* primer was present in all studied populations, it is not clear whether it is distributed in the same ratio among all *H. schachtii* populations. The standard curve should be constructed for certain nematode populations.

**REFERENCES**