## RESEARCH NOTE/NOTA DE INVESTIGACIÓN

# HOST STATUS AND HATCHING EFFECT OF HEMP (*CANNABIS SATIVA*) AND TEFF (*ERAGROSTIS TEF*) ON *GLOBODERA PALLIDA*

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## ABSTRACT

Hickman, P., L. Schulz, and L. M. Dandurand. 2023. Host status and hatching effect of hemp (*Cannabis sativa*) and teff (*Eragrostis tef*) on *Globodera pallida*. Nematropica 54:15-21.

The potato cyst nematode, *Globodera pallida*, is a regulated pest found in the United States only in the potato-growing areas of Idaho. Growers are interested in producing hemp (*Cannabis sativa*) and teff (*Eragrostis tef*) in *G. pallida*-infested fields. However, the host status of these two crops for *G. pallida* has not yet been evaluated. There is also interest in trap crops to help control *G. pallida*, which must be nonhosts that induce hatch of the nematode. This study investigated host status and hatching effect of hemp and teff on *G. pallida* through *in vivo* greenhouse experiments and *in vitro* assays. Both hemp and teff were nonhosts that did not induce hatch of *G. pallida*. Based on these results, hemp and teff are not trap crops for *G. pallida* but can be grown in infested fields because they are nonhosts.

*Key words: Cannabis sativa*, *Eragrostis tef*, *Globodera pallida*, hemp, potato cyst nematode, teff

## RESUMEN

Hickman, P., L. Schulz, and L. M. Dandurand. 2023. Estado del huésped y efecto del cáñamo (*Cannabis sativa*) y teff (*Eragrostis tef*) sobre la eclosión de *Globodera pallida*. Nematropica 54: 15-21.

El nematodo del quiste de la papa (PCN), *Globodera pallida*, es una plaga regulada en los Estados Unidos en Idaho. Los agricultores están interesados en producir cáñamo (*Cannabis sativa*) y teff (*Eragrostis tef*) en la región de la infestación. El estado del huésped de estos cultivos para PCN aún no ha sido evaluado. También existe interés en cultivos trampa para ayudar a controlar PCN, los cuales causen la eclosión del nematodo, pero que no sean su huésped. Este estudio investigó el estado del huésped y el efecto de estos cultivos en la eclosión de *G. pallida* a través de experimentos *in vivo* en invernadero y ensayos *in vitro*. Se determinó que el cáñamo y el teff no son huéspedes y no causan la eclosión de *G. pallida*. Según estos resultados, cáñamo y teff se pueden cultivar en campos infestados porque no son huéspedes, pero no serían cultivos trampa para PCN.

*Palabras clave: Cannabis sativa*, cáñamo, *Eragrostis tef*, *Globodera pallida*, nematodo del quiste de la papa, teff

*Globodera pallida* (Stone) Behrens, a potato cyst nematode, is a regulated pest of potato in the United States. It originated in the Andes but has become widespread in many potato-growing regions throughout the world. *Globodera pallida* was first reported in Idaho in 2006 (Skantar *et al*.,

2007). USDA-APHIS regulates *G. pallida*, and in 2023, APHIS reported 2,658 ha of regulated land in Idaho with 1,433 ha infested (USDA-APHIS, 2023). *Globodera pallida* is a serious threat to the potato industry with up to 80% yield loss predicted when infestations are severe (Contina *et al*., 2019). In the United States, Idaho is the top potato-producing state (USDA-NASS, 2023a). Based on 2016 data, *G. pallida* was estimated to cause a net annual loss of $25.56 million to the Idaho state economy even when other crops such as wheat or barley are produced to offset losses (Koirala *et al*., 2020).

*Globodera pallida* is a challenge to control because encysted eggs can remain viable in the soil in the absence of a host for decades (Turner, 1996). Second-stage juveniles (J2) require a hatching factor, which is present in root exudates, to trigger hatch (Masler and Perry, 2018). In the absence of the hatching factor, the nematode remains dormant in the soil as encysted eggs. Plant species producing *G. pallida* hatching factors are typically closely related to the primary host potato and in the Solanaceae family, but there are some exceptions such as quinoa and lupine (Franco *et al*., 1999, Scholte, 2000; Franco, 2003; Ngala *et al*., 2021). Idaho growers with infested acreage are interested in trap crops to help control *G. pallida*, in particular, crops that take advantage of hatching factors produced by nonhosts that prevent reproduction of the nematode. Hatched J2 are highly vulnerable without a host and quickly die (Storey, 1984). To determine the impact of a crop on the nematode and its potential usefulness as a trap crop for *G. pallida*, it is important to determine the host status and its ability to induce hatch. Once this is determined, then the crop may be useful to mitigate the impact of *G. pallida* in infested land where potato cannot be grown. The objectives of this study were to determine if hemp and teff are nonhosts of *G. pallida* and assess potential hatching effect for application as trap crops.

Production of hemp, *Cannabis sativa* L., in the United States has become more popular in recent years as restrictions have been lifted. In 2022, all U.S. industrial hemp was valued at $238 million with Idaho producing about 180 ha or 2% of acreage (USDA-NASS, 2023b). Hemp has been found to be parasitized by several plant-parasitic nematodes. A few *Meloidogyne* spp. have been reported to vary in degree of reproduction on hemp (Bernard *et al*., 2022). Hemp was found to be resistant to *M. chitwoodi* (Kok *et al*., 1994) suggesting that hemp could help suppress the nematode, while Ren *et al*. (2021) found hemp to be susceptible to *M. enterolobii*. Hemp has not yet been found to be a host for the highly specific cyst nematodes, such as those in genus *Globodera* (Bernard *et al*., 2022). Numerous plant-parasitic nematodes were found to be associated with hemp in Florida including reniform (*Rotylenchus reniformis*), root-knot (*Meloidogyne* spp.), spiral (*Helicotylenchus* spp.), stunt (*Tylenchorhynchus* spp.), ring (Criconematidae), stubby root (*Nanidorus minor*), and sting (*Belonolaimus longicaduatus*) (Desaeger *et al*., 2023). *Pratylenchus penetrans*, the root-lesion nematode, was recently confirmed to parasitize hemp (Núñez Rodríguez *et al*., 2023). Another study in the Netherlands found that hemp increased population densities of *P. penetrans* (Kok *et al*., 1994). There is also evidence that hemp as a soil amendment is antagonistic to plant-parasitic nematodes. Hemp leaves incorporated into the soil were found to reduce root galling and egg masses of *M. javanica* in peach tree roots (Saeed *et al*., 2021) and of *M. incognita* in cucumber (Kayani *et al*., 2012).

Teff, *Eragrostis tef* (Zucc.) Trotter, is an ancient grain that has been cultivated for thousands of years for grain and for forage. The majority of global teff production occurs in Ethiopia, its country of origin (Barretto *et al*., 2021). Production of teff has expanded in recent years due to more widely recognized nutritional benefits (Barretto *et al*., 2021). In the U.S., teff production has increased and is primarily grown as a livestock forage (Roseberg *et al*., 2005; Barretto *et al*., 2021). The interaction of teff with plant-parasitic nematodes has not been documented.

Greenhouse experiments and *in vitro* assays were conducted in summer 2022 to evaluate host status and hatching effect of teff and hemp on *G. pallida*. The *G. pallida* population used in the experiments was originally obtained from infested fields in Shelley, ID. The identity was confirmed by morphological and molecular methods (Skantar *et al*., 2007). The nematode was reared on the susceptible potato 'Désirée' or 'Russet Burbank' under greenhouse conditions at 18°C and 16-hr light:8-hr dark photoperiod for 16 wk before cysts were recovered. Cysts were recovered from soil by elutriator extraction and stored at 4°C until experimental use (USDA-APHIS, 2009). Cysts used in these experiments had been stored at 4°C

since fall 2021.

A host assay was performed in the greenhouse to determine whether teff and hemp are hosts to *G. pallida*. Sterile 10-cm-diam. terra cotta pots containing 500 g soil were inoculated with 10 *G. pallida* cysts to achieve an initial inoculation density of 5 eggs/g soil. Soil consisted of a 2:1 mix of Lane Mountain 20/30 industrial silica sand to Mission-series silt loam soil (University of Idaho Sandpoint Organic Agriculture Center, Sandpoint, ID) that had been dried and sieved through a 5-mm mesh (Dandurand and Knudsen, 2016). Soil was autoclaved twice for 90 min at 121°C with a 48-hour rest between cycles prior to use. For inoculation, cysts were sealed within sterile 6-cm$^2$ nylon mesh (McMaster Carr, Elmhurst, IL) bags with 250-µm opening, with edges sealed by a hand sealer (ULINE Tabletop Impulse Sealer, Pleasant Prairie, WI). Prior to inoculation, cysts were surface sterilized in 0.3% hypochlorous bleach for 5 min followed by five thorough rinses with sterile deionized water (Nour *et al*., 2003).

Teff 'Charger' (Alforex Seeds, Jordan, MN) and fiber hemp 'Yuma' (IND Hemp, Fort Benton, MT) were planted as seed and thinned to one plant per pot following emergence. Susceptible potatoes 'Russet Burbank' and 'Désirée' were included as positive controls and were transplanted as 4-wk-old tissue culture plantlets grown in standard media (Murashige and Skoog, 1962). Cyst bags were placed in the pot in the anticipated root zone of the plant for all when hemp and teff were seeded. Potato tissue cultures were transplanted when hemp and teff emerged. Bare soil unplanted pots were included as negative controls. Six replicate pots were grown in randomized complete block design for 10 wk. Greenhouse temperature was maintained between 16°C and 20°C with 60% relative humidity and a 16-hr light:8-hr dark photoperiod. Pots were watered daily to maintain soil moisture. Osmocote slow-release fertilizer (The Scotts Company, Marysville, OH) was applied at planting at the recommended label rate. Jack's Classic All Purpose 20-20-20 water soluble fertilizer (JR Peters Inc., Allentown, PA) was applied weekly at the recommended label rate. Bioworks SuffOil-X horticultural oil (Bioworks, Victor, NY) was applied weekly. Upon experiment termination, above-soil plant material was removed. Soil and root samples were dried for two weeks in pots and then collected for cyst extraction. Progeny cysts were extracted using the elutriator system (USDA-APHIS, 2009), then floated in acetone to further remove debris (Brodie *et al*., 1976). Samples were then examined under a dissecting microscope (Leica M80, Leica Microsystems, Wetzlar, Germany) where progeny cysts were counted.

Cyst bags used in original inoculation of the host assay were recovered and surface sterilized according to Nour *et al*. (2003). Four cysts per replicate were crushed in 100 ql sterile deionized water in individual wells of a 96-well plate (VWR, Radnor, PA). Eggs were counted under an inverted microscope (Leica DMi1). Average remaining viable encysted eggs were determined by the acridine orange staining method (Pillai and Dandurand, 2019). Non-viable eggs were counted with an inverted fluorescent microscope (Leica DMi8). Remaining viable eggs were calculated as total eggs – stained nonviable eggs. An additional four cysts per replicate were also used in a hatching assay adapted from Dandurand *et al*. (2017). Cysts were hydrated for 48 hr in 75µg/mL gentamicin then crushed and exposed to 100 µl potato root exudates (PRD). The percentage hatch rate of remaining encysted eggs for each cyst was determined after 2 wk of exposure to PRD.

*In vitro* hatch assays were performed using root exudates collected after 4 wk of growth. Plants for root exudate collection were grown in sterile 15-cm-diam. terra cotta pots containing 1,200 g sterilized 2:1 sand to soil mix as used in the host assay. Four replicate pots of the following treatments were grown in the aforementioned greenhouse conditions: teff, hemp, unplanted bare soil, and susceptible potato 'Désirée'. Root exudates were collected by leaching plant exudates from soil with water, modified from Widdowson and Wiltshire (1958). At 4 wk of growth, soil was saturated with deionized water 2 hr prior to exudate collection. Approximately 30 mL of root exudates per pot were collected by slowly adding deionized water to the pots until it flowed through into a collection cup. Exudates collected from each pot were kept as separate samples throughout this process to maintain treatment replicates. The exudate samples were then vacuum filtered first through a 0.45-qm-pore filter (Corning, Corning, NY) to remove soil particles and debris, and then through a 0.22-qm-pore filter to remove microbes (Corning). The resulting root exudates were frozen at -20°C until use.

The hatch assays utilized cysts of the Idaho *G.*

*pallida* population as described above. Cysts were surface sterilized in 0.3% hypochlorous bleach for 5 min before being thoroughly rinsed five times in sterile deionized water (Nour *et al*., 2003). Hatch assays were adapted from Dandurand *et al*. (2017) and performed in 96-well plates in which each well contained 200 µl of exudate with 75 µg/mL gentamicin and one cyst. Cysts were incubated at 18°C. After 3 wk incubation, emerged *G. pallida* J2 were counted using an inverted microscope (Leica DMi1). Cysts were then crushed and remaining encysted eggs were counted. There were eight wells serving as technical replicates per replicate of diffusate. The experiment was repeated. Percentage egg hatch was calculated as: (number of hatched J2)/ (initial eggs) x 100.

Statistical analysis was performed using the SAS statistical package (SAS Institute Inc., Cary, NC). Percentage hatch, progeny cysts, and egg densities were analyzed with a generalized mixed linear model (PROC GLIMMIX) and mean separation by least squares means. To meet the assumptions of a normal distribution, the host assay progeny cyst data was log10 (x +1) transformed prior to analysis.

No progeny cysts were found in the teff, hemp, or bare soil treatments in the host assay experiment, whereas many progeny cysts were extracted from the susceptible potato treatments ($P < 0.0001$) (Table 1). Based on these findings, and that published literature indicates that only solanaceous plants are hosts for *G. pallida* (Sullivan *et al*., 2007), we conclude that teff and hemp are nonhosts for *G. pallida*. The host assay also showed the susceptible potato control caused an 86 to 92% decrease in viable encysted eggs compared to bare soil, while the viability of the remaining population of encysted eggs for teff and hemp were not different than those from the bare soil control ($P < 0.0001$) (Table 1). Hatch of remaining eggs following the host assay was over 50% higher for bare soil, teff, and hemp compared to the encysted eggs from the susceptible potato control ($P < 0.0001$) (Table 1). This indicates that like the bare soil, teff, and hemp did not cause significant hatch of *G. pallida*.

The *in vitro* hatching assays using root exudates from each crop further investigated the potential hatching effect of hemp and teff and confirmed the non-host status of these two crop species. While potato induced 65% of the eggs to hatch, teff and hemp induced the same level of hatch as the bare soil control; 2%, 6%, and 5% egg hatch, respectively. The hatch rate induced by potato was significantly greater than that induced by teff, hemp, or bare soil ($P < 0.0001$) (Fig. 1A), but hatch rate did not significantly differ among hemp, teff, or bare soil exudates. When repeated, similar results were observed. Teff, hemp, or bare soil exudates collected in the second trial induced a similar hatch rate, which was not significantly different, while potato induced significantly greater hatch than teff, hemp, or bare soil diffusate ($P = 0.0186$) (Fig. 1B). This study confirms the nonhost status of teff and hemp. However, these two crops are not trap crops for *G. pallida* as they will not decrease population densities because their root exudates do not induce hatch. The data presented here suggests that teff and hemp planted in infested Idaho fields would not have a significant impact on *G. pallida* but could be planted in infested fields as nonhost crops.

Table 1. *Globodera pallida* host assay remaining viable encysted eggs recovered from cyst bags, hatch of remaining encysted eggs in potato root diffusate (PRD), progeny cysts, and determined host status for *G. pallida*.

| Treatment | Remaining viable eggs/cyst | Hatch of remaining eggs[y] | Progeny cysts | Host status for *Globodera pallida* |
|---|---|---|---|---|
| Bare soil (fallow) | 151.4 a[z] | 68.8% a | 0 c | Nonhost |
| Teff | 170.0 a | 59.8% a | 0 c | Nonhost |
| Hemp | 166.8 a | 58.3% a | 0 c | Nonhost |
| Susceptible potato 'Désirée' | 12.8 b | 8.0% b | 88 a | Host |
| Susceptible potato 'Russet Burbank' | 21.1 b | 17.6% b | 22 b | Host |

[y]Hatch of remaining encysted eggs exposed to potato root diffusate for two weeks.

[z]Data presented are means of six replicates. Significant differences are denoted by different letters in the columns based on least squares means at $\alpha = 0.05$.

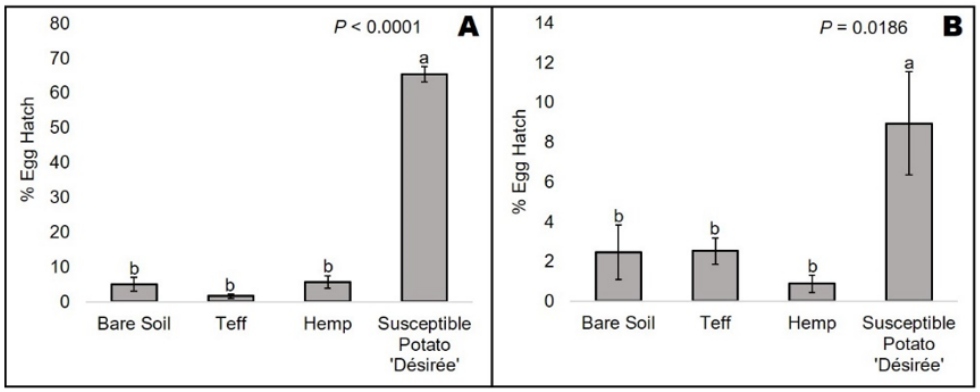


Fig. 1. Mean percentage egg hatch of *Globodera pallida* determined 3 wk after root exudate application for trial 1 (A) and trial 2 (B). Standard error of the means is indicated by the bars. Different letters indicate significantly different means based on least squares means at $\alpha = 0.05$.

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