# Early Crop Root Destruction for Management of Tobacco Cyst Nematodes

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*Abstract:* Prompt tillage after crop harvest was investigated as a cultural control for the tobacco cyst nematode, *Globodera tabacum tabacum*, on stalk-cut broadleaf cigar wrapper tobacco. Stalk stumps and roots remaining after harvest were destroyed by tilling immediately or from 2 to 6 wk after harvest in field experiments over 4 yr. Cyst nematode Pf/Pi ratios ranged from 0.65 to 1.62 when plants were tilled immediately after harvest and 1.13 to 5.88 when tillage was delayed. Nematode population development was monitored by inoculating plants in pots placed in fields with J2 in eggs and sampling over time (8 to 18 wk). Three generations per year were observed, and *G. t. tabacum* generation time was as short as 6 wk for each generation. Destroying stalks and root systems remaining after harvesting stalk-cut broadleaf cigar wrapper tobacco removes the host to preclude development of nematodes at the end of the second and entire third generation. Early tillage resulted in consistently lower tobacco cyst nematode populations than allowing viable roots to remain in fields for an additional 8 to 18 wk. This management tactic reduces the need for nematicide application to slow nematode population increases over time and can reduce losses due to infection by *G. t. tabacum.*

*Key words:* Cultural control, *Globodera tabacum tabacum*, management, *Nicotiana tabacum*, Nemacur, root destruction, tillage, tobacco, tobacco cyst nematode.

The tobacco cyst nematode, *Globodera tabacum tabacum* (Lownsbery & Lownsbery) Stone, is an economically important pathogen of cigar wrapper tobacco (*Nicotiana tabacum* L.) types in the Connecticut River Valley of Connecticut and Massachusetts. The nematode can cause stunting and yield loss, and reduce leaf quality of shade-grown (Lownsbery and Peters, 1955; LaMondia, 1995) and stalk-cut broadleaf cigar wrapper tobacco (LaMondia, 2002) as well as increase the incidence and severity of Fusarium wilt of broadleaf tobacco (LaMondia and Taylor, 1987). In order to reduce losses due to nematode infection, pre-plant densities of *G. tabacum* in soil need to be maintained below damaging levels.

Cyst nematode management options in tobacco can be difficult, expensive and of limited availability. The nematicide oxamyl is ineffective against cyst nematodes at currently registered rates (9 liters/ha), and the nematicide fenamiphos, while more efficacious, is no longer available. Rotation reduces nematode densities by only about 20% over one year, and fumigation can be expensive and requires specialized equipment. It is important, then, to develop practices that reduce pre-plant nematode densities for the next cropping season and limit increases in cyst nematode populations as much as possible. Broadleaf tobacco is a stalk-cut tobacco type, leaving 1 to 5 cm of a stalk 'stump' with viable roots after harvest, upon which nematodes can continue to feed and develop, often until the ground freezes, one or two months after harvest.

An early cultural control method for root-knot nematodes in tobacco and tomatoes (Atkinson, 1889; Todd, 1979) involved destruction of crop roots as soon after harvest as possible. This practice limits nematode re-

production and exposes eggs and juveniles to drying, further reducing inoculum for the next crop. Although cyst nematodes may be protected from drying, early crop destruction should limit further increases in nematode populations after crop harvest.

The objective of this research was to determine the effect of crop residue destruction immediately after harvest on cyst nematode populations compared to tillage at a later date.

## Materials and Methods

The effects of tillage to destroy stalk stumps and associated host plant roots remaining after stalk cutting and limit additional tobacco cyst nematode reproduction and development were determined in field experiments conducted in 1997, 1999, 2001 and 2002. Additionally, nematode development was independently monitored on host plants placed into field soils in pots in 2001 and 2002. In all small-plot experiments, one- or two-row plots were transplanted with 10 plants/row of Connecticut broadleaf tobacco cultivar 'C9'. Plant spacing was 1 m between rows with plants 0.6 m apart within rows. Cyst nematode densities were determined by sampling before planting, at harvest, and at the conclusion of the experiment after all plots had been tilled. The soil (tested in 1997) was a Merrimac fine sandy loam (Entic Haplorthod; 71.8% sand, 23.0% silt, 5.2% clay; 3.0% organic matter; pH was adjusted with lime annually and was 6.0, 5.8, 5.9 and 5.9 in 1997, 1999, 2001 and 2002, respectively). Fertilizer and pesticide applications were performed as per commercial practice. Each year, all plots received nitrogen incorporated pre-plant (224 kg/ha) and nitrogen side-dressed (56 kg/ha) at 24 d after transplanting (5.9–2.8–6.1 N-P-K cottonseed meal base). Pre-plant applications of chlorpyrifos (7.0 liters Lorsban 4E/ha; Dow AgroSciences Indianapolis, IN) and mefanoxam (1.2 liters Ridomil Gold EC/ha; Syngenta Crop Protection, Greensboro, NC) were incorporated in all plots 24 hr before transplanting. Foliar in-

sects were controlled by acephate (1 kg a.i./ha) applied to all plots as needed.

*Globodera tabacum tabacum* populations were estimated each year before transplanting and again after harvest. Soil was sampled by removing 10 2.5-cm-diam. cores to 15-cm-deep per plant row in each plot. All soil samples were air dried, and *G. t. tabacum* cysts were extracted from the soil with a modified Fenwick can and crushed in water. Two aliquots of nematodes in tap water suspension were counted to determine the number of free *G. t. tabacum* second-stage juveniles (J2) and eggs per cubic centimeter of soil.

*1997 Field Experiment:* Initial soil samples to determine Pi were taken, and plants transplanted to 30 plots (15 replicated paired-row plots) on 10 June. Plants were stalk-cut to remove shoots on 20 August, and paired rows were either tilled immediately after harvest or 15 d later on 4 September. Final nematode densities were determined from soil samples taken on 7 November. Data were analyzed by paired *t*-tests.

*1999 Field Experiment:* The experiment was designed as a 2×2 factorial with nematicide and tillage timing treatments replicated 8 times. Thirty-two single-row plots were sampled for Pi and either treated with 18 liters/ha Nemacur 3ES (Bayer CropSciences, Research Triangle Park, NC) in 200 liters/ha water on 4 June 1999 or left untreated. Tobacco was transplanted to plots on 7 June and harvested on 9 August. Plots were tilled either 10 August or 14 September, and nematode Pf samples taken on 15 September. Data were analyzed by Analysis of Variance (ANOVA).

*2001 Field Experiment:* Eight paired-row plots were sampled for Pi, and plants were transplanted on 13 June. There were four replicate plots, each with one row tilled immediately after harvest on 17 August and the second row tilled on either 10 or 27 September. Final nematode samples were taken on 5 October. Data were analyzed by ANOVA.

*2002 Field Experiment:* Eight paired-row plots were sampled for Pi, and plants were transplanted on 18 June. There were four replicate plots each, with one row tilled immediately after harvest on 12 August and the second row tilled on either 4 or 24 September. Final nematode samples were taken on 25 September.

*2001 and 2002 Pot Experiments:* Nematode population development over time was monitored over the 2001 and 2002 seasons by placing pasteurized soil (heated to 82°C for 2 hr) from an adjacent field with the same soil type not planted to tobacco within the last 30 years and free of cyst nematodes in 24 3.8-liter plastic pots and submerging the pots in field soils within 50 m of the field experiments in an untilled area where tobacco had not been grown and *G. t. tabacum* cysts were absent or undetectable. The nematode-susceptible tobacco cultivar '8212' was transplanted into pots on 15 May 2001 and 24 May 2002. *Globodera t. tabacum* cysts were crushed and sieved to allow inoculation of all pots with

20,000 J2 in eggs/pot, without cysts or large cyst fragments. Inoculation occurred on 29 May 2001 and 29 May 2002. Four replicate pots were removed from soil 8, 10, 12, 14, 16 or 18 wk after inoculation. Shoots were removed, and the pot contents (including roots) were mixed prior to taking a 50-cm$^3$ subsample for sugar centrifugation. The remainder of the soil was dried, and cysts were extracted from the entire sample by a modified Fenwick can after the last sample had dried (about 20 wk after inoculation).

## Results

*1997 Field Experiment:* Final populations of *G. t. tabacum* increased by an additional 55% when stalks and roots left after broadleaf tobacco was harvested were not tilled until 15 d later, rather than immediately after harvest ($P = 0.03$) (Table 1). There were no differences in initial population densities ($P = 0.05$), but Pf/Pi ratios were 1.20 for immediate tillage vs. 1.94 for tillage after 15 d ($P = 0.02$).

*1999 Field Experiment:* Cyst nematodes increased more over the season in non-treated plots compared to plots treated with Nemacur ($P = 0.01$) (Table 2). Early tillage reduced *G. t. tabacum* population growth compared to waiting 5 wk after harvest to till stalks and roots, whether Nemacur was applied or not ($P = 0.02$). Early tillage alone was not different than Nemacur application and late tillage in maintaining cyst nematode densities at lower levels ($P = 0.05$).

*2001 Field Experiment:* At low initial *G. t. tabacum* densities (less than 20% of the damage level of 50 J2/cm$^3$ soil), tillage immediately after harvest prevented populations from increasing significantly (Table 3), whereas waiting 3 to 6 wk after stalk-cutting allowed substantial increases in cyst nematode numbers ($P = 0.001$).

*2002 Field Experiment:* Cyst nematode population growth as determined by Pf/Pi ratio was lower when the 2001 experiment was repeated in soils with higher nematode densities, but results were similar (Table 4). Early tillage on the same day as harvest decreased nematode densities compared to waiting 3 to 6 wk after harvest before tilling-in roots.

*2001 and 2002 Pot Experiments:* Population develop-

TABLE 1. Timing of stalk and root destruction by tillage after stalk-cut broadleaf tobacco harvest on *Globodera tabacum tabacum* populations, 1997.

| Tillage (days after harvest) | Pf[a] | Pf/Pi[b] |
|---|---|---|
| 0 | 87.4 | 1.20 |
| 15 | 136.0 | 1.94 |
| *P* = | 0.03[c] | 0.02 |

[a] *G. t tabacum* population expressed as encysted J2 per cm$^3$ soil. Initial nematode densities averaged 70 J2/cm$^3$ soil in 15 replicate paired row plots.

[b] Ratio of final to initial *G. t tabacum* densities in soil. Pf/Pi data were square root transformed prior to analysis.

[c] Data were analyzed by the Paired *t*-test.

TABLE 2. The effect of application of the nematicide fenamiphos (18 liters/ha Nemacur 3ES) and timing of stalk and root destruction by tillage after stalk-cut broadleaf tobacco harvest on *Globodera tabacum tabacum* populations, 1999.

| Nematicide | Tillage timing | Pf/Pi ratio |
|---|---|---|
| Fenamiphos | Immediately after harves | 0.59 |
| Fenamiphos | 5 weeks after harvest | 1.32 |
| Nontreated | Immediately after harvest | 1.62 |
| Nontreated | 5 weeks after harvest | 4.87 |
| Factor | Significance (*P* =) | |
| Nematicide | 0.01 | |
| Tillage | 0.02 | |
| Nematicide × Tillage | ns | |

Data were square root transformed prior to analysis by Analysis of Variance. Initial nematode densities averaged 13.56 J2/cm³ soil. There were eight replicate plots in a factorial design.

ment of *G. t. tabacum* was investigated in pots placed into the field to allow development of a single cohort in pots under field conditions similar to the nearby field plots (Fig. 1). Numbers of hatched infective J2 free in soil and numbers of encysted J2 present after 20 wk were determined for pots with plants destroyed at 8 to 18 wk after inoculation and placement in the field. The numbers of infective J2 in soil peaked at 10 wk (J2 hatched from the first generation) and again at 16 to 18 wk after inoculation week (J2 hatched from the second generation). Increases in encysted J2 occurred when females matured and were consistent with an egg to egg generation time of about 5 to 6 wk.

## Discussion

Destruction of crop plants and residues that remain after harvest as a cultural control of nematode pathogens may have two types of impacts on the target nematodes (Barker and Lucas, 1984). First, removal or destruction of living plant roots can reduce the length of time that the parasites can feed on the roots, which may lead to increased population densities. Second, the tillage involved in root destruction may expose nematodes and eggs to the sun and air, which dries the soil more quickly and results in the death of nematodes at different life stages. Todd (1979) adapted the concept of

TABLE 3. Timing of stalk and root destruction by tillage after stalk-cut broadleaf tobacco harvest on *Globodera tabacum tabacum* populations, 2001.

| Tillage (weeks after harvest) | Pi[a] | Pf | Pf/Pi[b] |
|---|---|---|---|
| 0 | 4.4 | 4.6 | 1.03 |
| 3 | 6.3 | 24.4 | 5.42 |
| 6 | 8.3 | 24.8 | 5.88 |
| *P* = | ns[c] | 0.03 | 0.001 |

[a] *G. t tabacum* Pi and Pf populations expressed as encysted J2 per cm³ soil.
[b] Ratio of final to initial *G. t tabacum* densities in soil. Pf/Pi data were square root transformed prior to analysis. There were four replicate plots of each treatment.
[c] Data were analyzed by Analysis of Variance.

TABLE 4. Timing of stalk and root destruction by tillage after stalk-cut broadleaf tobacco harvest on *Globodera tabacum tabacum* populations, 2002.

| Tillage (weeks after harvest) | Pi[a] | Pf | Pf/Pi[b] |
|---|---|---|---|
| 0 | 88.9 | 55.1 | 0.65 |
| 3 | 114.3 | 121.6 | 1.13 |
| 6 | 125.5 | 131.8 | 1.02 |
| *P* = | ns[c] | 0.04 | 0.03 |

[a] *G. t tabacum* Pi and Pf populations expressed as encysted J2 per cm³ soil.
[b] Ratio of final to initial *G. t tabacum* densities in soil. Pf/Pi data were square root transformed prior to analysis. There were four replicate plots of each treatment.
[c] Data were analyzed by Analysis of Variance.

crop and root destruction proposed in 1889 by Atkinson to control nine pests in tobacco, including root-knot nematodes. The efficacy of this approach may depend on the activity of the life stages of the particular nematode target in or on the host plant and the specificity of the target nematode for a particular host. Tobacco roots may survive for quite some time after harvest, and nematodes may still be active for much of this period.

*Globodera tabacum tabacum* Pf/Pi ratios ranged from 0.65 to 1.62 when plants were tilled immediately after harvest and from 1.13 to 5.88 when tillage was delayed from 2 to 6 weeks in these experiments. Nematode population development was monitored by inoculating
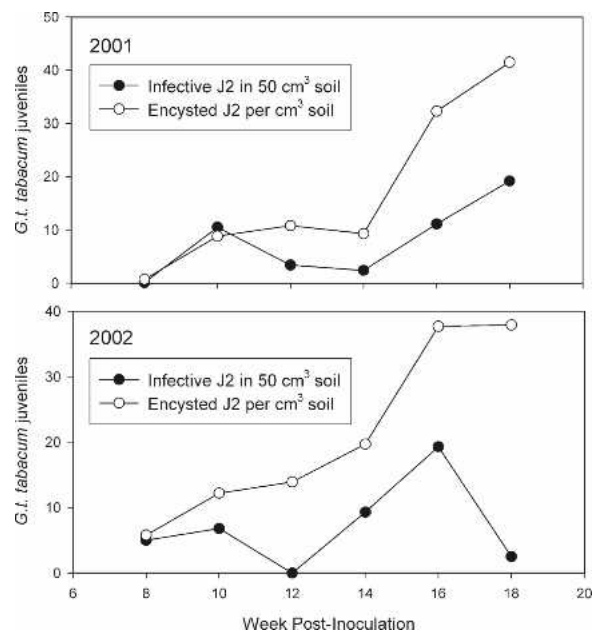


FIG. 1. The number of infective second-stage *Globodera tabacum tabacum* juveniles recovered from 50 cm³ soil from 8 to 18 wk after inoculation and encysted second-stage juveniles in eggs per cm³ soil present in 3.8-liter pots after 20 wk when plants were destroyed at 8 to 18 wk after inoculation. Plants were inoculated with 20,000 J2/pot, and four pots were destructively sampled at each date. Infective J2 were recovered by sugar centrifugation at the time of plant destruction, and encysted J2 were extracted by means of a modified Fenwick can from soil dried after plant destruction and held until 20 wk after inoculation. Data are shown for 2001 and 2002.

plants in pots placed into field soils with J2 in eggs, and then sampling over time (8 to 18 weeks). Three generations per year were observed, and *G. t. tabacum* generation time was as short as 6 weeks. The first generation was not sampled in these experiments.

Broadleaf tobacco in Connecticut is harvested after 8 to 10 weeks in the field, and cyst nematodes may remain active for an additional 8 to 10 weeks or more. The roots of tobacco planted in May or early June and harvested in August may not die due to frost until November. Our current results and previous data (LaMondia, 1996) indicate that *G. t. tabacum* completes a first generation at about 6 weeks after transplanting and a second generation about 12 weeks after transplanting. A third generation may be completed in an additional 6 weeks. Destruction of plant roots by tillage immediately after harvest (8–10 weeks after planting) may limit development of the second generation already in roots and preclude a third generation, resulting in smaller population increases relative to those present after tillage occurring two or more weeks later. Those motile juveniles able to move to other plants have a limited host range and do not increase on the small grains typically grown as fall/winter cover crops.

Tobacco cyst nematodes overwinter as unhatched J2 in eggs in cysts. While tillage likely disrupts the development of immature nematodes in roots, nematode mortality due to exposure to drying and sunlight is less likely than for root-knot nematodes, as the eggs are protected by the cyst and likely more difficult to kill than root-knot nematodes. Drying may be more effective against root-knot (Todd, 1979) than cyst nematodes, as we dry soil for cyst nematode extraction and routinely store air-dried *G. t. tabacum* cysts for up to a year with little mortality (unpubl).

Similar cultural management tactics have had variable success against other nematodes. Cotton crop destruction after harvest has not had consistent results on *Hoplolaimus columbus* populations (Davis et al., 2000; Koenning et al., 2003) and did not increase yields in the next growing season. The lack of impact on *H. columbus* in the cotton system may be due to low soil temperatures late in the season and reduced nematode activity and reproduction at that time (Koenning et al., 2003). When temperatures were more favorable for nematode activity after crop harvest, as with greenhouse crops and with banana, results were more significant and consistent. Ornat et al. (1999) demonstrated that fallow and root destruction were more effective than fallow alone for control of *Meloidogyne arenaria* and *Pratylenchus neglectus* after greenhouse-grown green beans. Chabrier and Queneherve (2003) reported that increasing the speed of banana root death by application of glyphosate, in addition to mechanical breakdown rather than mechanical destruction alone, reduced *Radopholis similis* populations after fallow and increased yields in the next banana crop.

Our research has demonstrated that while tobacco cyst nematodes may complete up to three generations per year at roughly 6 week intervals, early tillage to destroy stalk 'stumps' and associated root systems remaining after harvest of stalk-cut, broadleaf cigar wrapper tobacco consistently results in lower tobacco cyst nematode populations than allowing plant stalks to remain and re-sprout, maintaining viable roots for months. This management tactic should reduce the need for nematicide application and reduce losses due to infection by *G. t. tabacum* in subsequent crops. Crop destruction after harvest should also reduce the inoculum of other pathogens such as Tobacco Mosaic and other viruses, as well as fungal pathogens such as *Peronospora tabacina* and *Fusarium oxysporum* for subsequent crops.

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