The mycophagous nematode *Panagrolaimus multidenatus* reduces the size of necrotic cankers caused by *Geosmithia morbida* in black walnut

Geoffrey M Williams
College of Natural Resources, University of Idaho, Moscow, ID 83844
Current Address: Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN 47907 (Ginzel Lab)

INTRODUCTION

- *Geosmithia morbida* Kolarik (Fig. 1) is the necrotic canker pathogen associated with Thousand Cankers Disease (TCD), a pest complex that has caused death of eastern black walnut (*Juglans nigra*) across the USA (Figs. 2a, b)

Fig. 1: *Geosmithia morbida* colonized agar plate

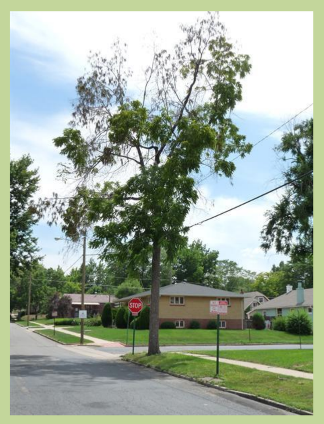

Fig. 2a: TCD-symptomatic tree

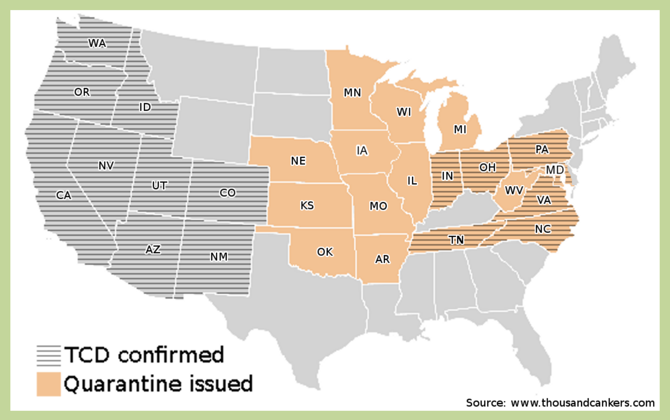

Fig. 2b: Current confirmed range of TCD and active quarantines in the USA

- *Panagrolaimus multidentatus* Ivanova det. Lynn Carta, USDA-APHIS (Figs. 3a, b) is a nematode that was isolated from crown branches of *J. nigra* growing in Moscow, Idaho

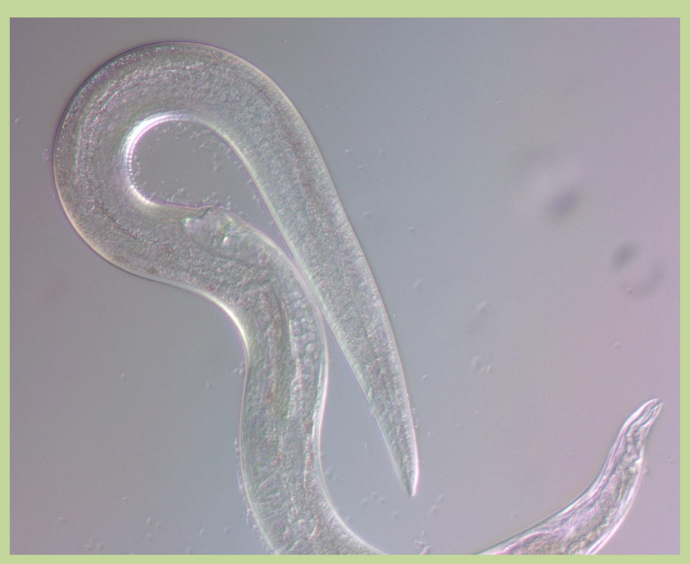

Fig. 3a: Female *P. multidentatus*

Fig. 3b: Male *P. multidentatus*

- Initial observations led to preliminary assays on the range of suitable fungi from walnut wood on which *P. multidentatus* could feed and complete its lifecycle, including *G. morbida*

Figure 4: *P. multidentatus* feeding on *G. morbida*

- After twenty days, an initial population of 20 *P. multidentatus* grew to 2000 on lab cultures of *G. morbida*, leaving scarce mycelium and no conidiophores remaining (Fig. 4)
- High population growth also occurred on *Epicoccum nigrum* and *Fusarium* sp.; completion of nematode lifecycle was confirmed on all three fungi through successive subcultures
- *P. multidentatus* was assayed for biocontrol potential *in situ* by co-inoculating branches of black walnut with *G. morbida*

OBJECTIVE

Determine the extent to which the walnut nematode *Panagrolaimus multidentatus* alters the growth of necrotic cankers caused by *Geosmithia morbida* on black walnut branches

METHODS

Study design and inoculation

- Study was conducted on 64 *J. nigra* of unknown genotype at a planting in Preston, Idaho (Fig. 5) owned and operated by Utah State University
- *P. multidentatus* were raised on an isolate of the benign *E. nigrum* from walnut wood and extracted with a Baermann funnel for the experiment

Fig. 5: Walnut planting where study was conducted

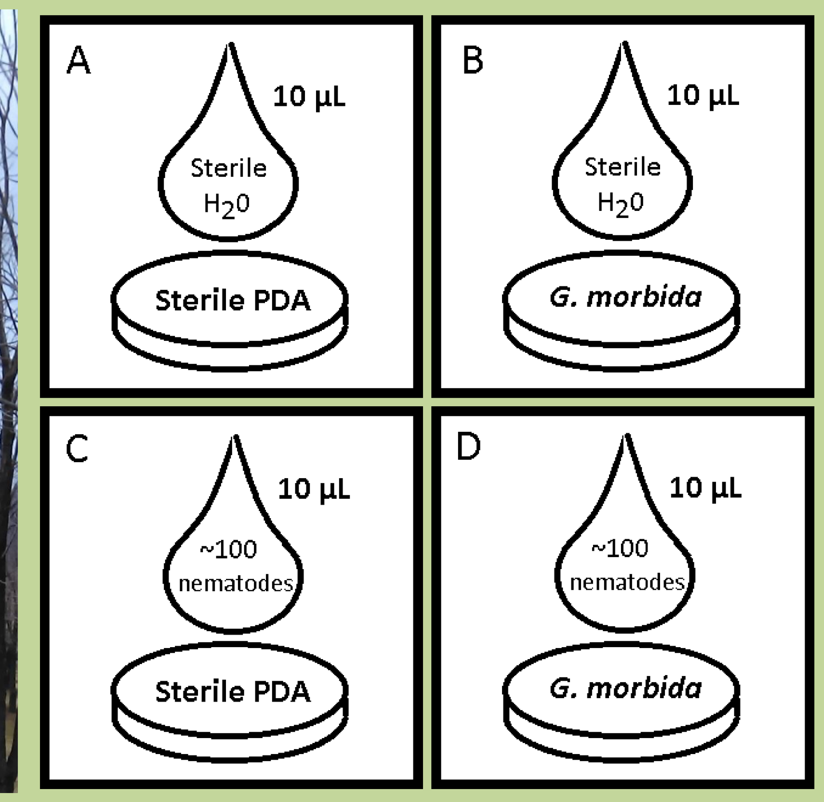

Fig 6: 2-by-2 factorial treatment design (A-D)

- In a two-by-two factorial design (Fig. 6), branches were inoculated with: (A) control, (B) *G. morbida*, (C) *P. multidentatus*, or (D) both
- Each tree received all four treatments, and each treatment was replicated twice on one branch per tree (Fig. 8)
- Bark was removed with a cork borer to expose phloem
- Agar plugs were placed in wound and then sealed
- Inoculations occurred soon after leaf emergence in June 2016

Data collection and analysis

- In November of 2016, branches were harvested; branch diameter and canker size (area of necrotic phloem) were measured in field

Fig. 7: Canker length and width were measured after removing bark to reveal phloem

- Bark was removed to reveal phloem and canker length (D1) and width (D2) were measured with calipers and recorded (Fig. 7)
- Canker area was calculated as elliptic area with $A = \frac{1}{4} \pi \times D1 \times D2$
- Data were analyzed using a simple linear model with branch diameter as a continuous covariate and tree as a blocking factor
- *G. morbida* (B) and *Panag + Geo* (D) treatments were compared
- Data were log-transformed to satisfy assumption of normality

RESULTS

Fig. 8: A replicate example

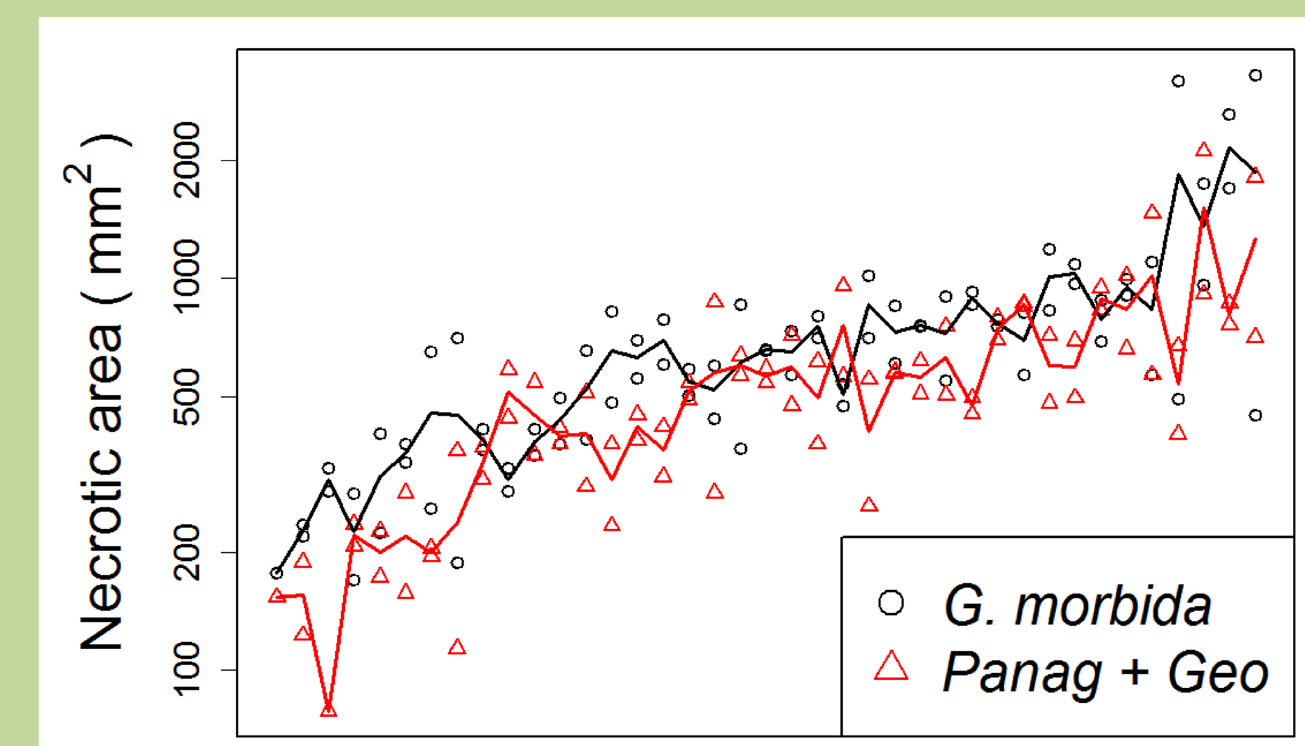

Fig. 9: Mean (lines) and individual (points) canker size by treatment, ordered by tree effect

- *Panag + Geo* treatment resulted in a 24.4% reduction in necrotic area compared to *G. morbida* across trees (Fig. 9)
- No necrosis in control or *P. multidentatus* treatments (Fig. 8)
- Wounds on *Panag + Geo* treatment appeared to heal (Fig. 7)
- Branch diameter did not have a significant effect ($R^2 < 0.01$)
- Tree effect was highly significant ($F = 7.68$; $p < 2.2E\text{-}16$)
- Treatment effect was highly significant ($F = 20$; $p < 1.67E\text{-}05$)
- Least square means for treatments (± SE):
  - *G. morbida*: $e^{6.38 \pm 0.04}$ = [564 mm^2, 616 mm^2]
  - *Panag + Geo*: $e^{6.10 \pm 0.04}$ = [427 mm^2 , 466 mm^2]

CONCLUSIONS

Panagrolaimus multidentatus, *Epicoccum nigrum*, associated waste products, and/or metabolites reduce growth of cankers caused by *Geosmithia morbida*

FUTURE DIRECTIONS

- Test *E. nigrum* and *P. multidentatus* separately to determine relative contribution to observed reductions in canker size
- Conduct reisolation assays to determine persistence of *P. multidentatus* in walnut branches
- Use image analysis to measure canker area with precision
- Repeat study over multiple seasons
- Sample black walnut for arboreal nematodes in native range

ACKNOWLEDGEMENTS

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