SCIENTIFIC NOTE

Mortality of *Metarhizium anisopliae*-infected wireworms (Coleoptera: Elateridae) and feeding on wheat seedlings are affected by wireworm weight

WILLEM G. VAN HERK[1] and ROBERT S. VERNON[1]

As some wireworm species are notorious pests of common wheat, *Triticum aestivum* (Vernon *et al*. 2009), a small study was conducted at the Pacific Agri-Food Research Centre (PARC) in Agassiz, BC in August 2009, to determine whether the number of germinating wheat seedlings (cv. AC Barrie) killed by the dusky wireworm, *Agriotes obscurus*, is affected by the number and size of the wireworms that seedlings are exposed to. An unexpected factor appeared at the end of the study in that many wireworms were infected with *Metarhizium anisopliae*, resulting in considerable mortality. This factor precluded us from meeting some of the initial objectives of the experiment, but still allowed us to determine if mortality of seedlings was affected by wireworm weight and number, and if mortality of the wireworms from *M. anisopliae* infection was affected by their weight.

We filled 160 500 ml circular (dia = 11 cm, height = 8 cm) plastic containers (Plastipak Industries, Inc., La Prairie, QC) with 500.0 (+/- 0.2) g of soil collected from a field at PARC, Agassiz in 2009. The soil was sieved through a 2 mm x 2 mm screen to remove rocks and organic material, made up to 20% moisture by weight, and homogenized. Containers with soil were placed in a walk-in cooler set at 15.0 +/-0.5°C to mimic soil temperature conditions in spring when wheat is normally planted. Wireworms were weighed individually on August 7, and 0-4 wireworms were placed in each container (Table 1). All wireworms were collected at PARC in April 2009 and stored in 40l Rubbermaid tubs without food, at 8-10°C, until 1 wk before the study, when tubs were brought up to room temperature and food baits (a cup of 100 ml moist vermiculite mixed with 10 ml wheat seed) placed inside. Only mobile wireworms, appearing healthy (Vernon *et al*. 2008) and actively feeding 2 to 3 d prior to placement in the containers were used for this study. Wireworms selected ranged considerably in weight (range: 6.6 to 46.4 mg; Table 1), but all wireworms in individual containers were similar in weight (within 5.0 mg), and an attempt was made to have an equal number of similar sized wireworms for each of the 1, 2, and 3 or 4 wireworm densities to determine the effect of wireworm weight on the number of wheat seedlings killed.

Two days after wireworms were placed in containers, 21 untreated wheat seeds were planted 2 cm deep in small pre-made holes. Seeds were spaced at equal distance (1.75 cm) from each other, in a 3, 5, 5, 5, 3-grid pattern. After planting, groups of eight containers were placed in 26 cm x 47 cm x 6 cm deep nursery flats (Eddi's Wholesale Garden Supplies, Ltd., Surrey, BC), 1.0l cold water added between the containers in the flat, and flats covered with 14 cm high transparent plastic domes (Eddi's Wholesale) to prevent desiccation of the upper layer of soil. After planting, containers were subjected to a 12:12 light:dark regimen.

Seedling emergence was first observed 5 d after planting, stand counts were conducted 8 d (when domes were permanently removed due to the length of plant shoots) and 15 d after planting. Wireworms were removed 25 d after planting and their health evaluated (Vernon *et al*. 2008). This revealed that only 123 of 255 larvae were alive, the rest having died from *Metarhizium* infection, most likely within the first two weeks of the study as evident from the extent of mould formation on the surface of the cadavers. Analysis of the proportion of wireworms dead in each

[1] Pacific AgriFood Research Centre, Agriculture and AgriFood Canada, P.O. Box 1000, V0M 1A0, Agassiz, British Columbia, Canada

Table 1.
Proportion of wheat seedlings dead or not emerging 15 days after planting. N = number of containers. Shown are least squares means and SE estimates calculated from ANCOVA; all least squares means are significantly different from 0 at P<0.0001. Numbers followed by different letters in columns are significantly different from each other at P<0.05, using a Tukey-Kramer adjustment.

No. of wireworms in container	N	Wireworm weight range (mg)	Model 1: All wireworms placed in containers	N	Model 2: Surviving wireworms alone
0	43		0.084(0.018)A	80	0.113 (0.009) A
1	43	6.6 - 46.4	0.101(0.012)A	50	0.151 (0.012) AB
2	36	8.6 - 42.3	0.166 (0.013) B	21	0.202 (0.018) BC
3	12	25.8 - 43.7	0.233 (0.023) BC	5	0.246 (0.037) BC
4	26	8.2 - 41.3	0.250 (0.014) C	4	0.313 (0.041) C
ANCOVA		*Flat:*	F=1.18, df=19,135, P=0.28		F=0.97, df=19,135, P=0.50
Statistics		*No. of wireworms:*	F=25.15, df=4,135, P<0.0001		F=11.51, df=4,135, P<0.0001
		Wireworm weight:	F=0.51, df=1,135, P=0.47		F=16.78, df=1,135, P<0.0001

container with ANCOVA (PROC GLM, SAS 9.1) with variable container flat and covariate average wireworm weight in containers, indicated that flat did not have a significant effect (F=0.91, df=19,96, P=0.57), but wireworm weight did (F=14.17, df=1,96, P=0.0003). Eliminating the variable flat from the analysis and regressing the proportion of wireworms dead to the average wireworm weight in each container produced the following model: Proportion dead = 0.107 + 0.015 × wireworm weight (SE = 0.099, 0.004; t=1.08, 4.42; P=0.28, <0.0001, respectively; model R^2 = 0.145), indicating that the proportion of wireworms dead increased with the average weight of wireworms in the container.

Considering the mortality of wireworms during the experiment, two separate analyses were conducted to determine the effect of wireworm number and weight on wheat seedling survival. In the first analysis, the proportion of wheat seedlings that did not emerge by 15 d after planting was evaluated with ANCOVA, with variables flat and the number of wireworms originally placed in the container, and the covariate average wireworm weight per container (Table 1, Model 1). Treating the number of wireworms in the container as a variable allowed us to calculate least squares means for the proportion of seedlings killed per wireworm density, and produced a similar model as when both wireworm number and average weight were included as covariates. The second analysis was similar, differing only in that the number of wireworms that survived was included. Both models indicated that the flat in which containers were placed did not have a significant effect on plant mortality (P>0.05; Table 1), and that the number of wireworms in the container was highly significant (P<0.0001), with the proportion of plants killed increasing with wireworm number (Table 1). The weight of wireworms in the container did not appear to significantly affect the number of plants killed if all wireworms placed in each container were considered. However, as heavier wireworms were more likely to die from *Metarhizium*, and mortality appeared to have occurred early in the study when the wheat plants were most susceptible to wireworm attack, this is probably a misleading conclusion. When only surviving wireworms are included in the analysis (Table 1, Model 2), it is apparent that heavier wireworms caused more damage than smaller ones. While this confirms the expectation that larger wireworms are more destructive to wheat seedlings than smaller ones, the finding that larger wireworms are more likely to die from *Metarhizium* than smaller wireworms is novel and of importance, as it suggests that using the fungus as a biological control agent for wireworms may be more effective for later than earlier instars. This relationship has apparently not been observed in wireworms before, and should be confirmed and explained with further study. As *Metarhizium* is commonly present in Agassiz soil, all locally collected larvae likely contain spores and an environmental trigger (e.g. temporary exposure to a high temperature) necessary to induce infection. Considering the LT50 of *A. obscurus* after *Metarhizium* infection, the infection seen here was likely triggered prior to wireworm placement in containers (Kabaluk and Ericsson 2007).

ACKNOWLEDGEMENTS

We thank our summer students, Selina McGinnis, Heike Kösterke, and Chantelle Harding for helping collect and weigh wireworms and plant wheat seeds.

REFERENCES

Kabaluk, J. T., and J. D. Ericsson. 2007. Environmental and behavioral constraints on the infection of wireworms by *Metarhizium anisopliae*. Environmental Entomology 36: 1415-1420.

Vernon, R.S., van Herk, W., Tolman, J., Saavedra, H.O., Clodius, M., and B. Gage. 2008. Transitional sublethal and lethal effects of insecticides after dermal exposures to five economic species of wireworms (Coleoptera: Elateridae). Journal of Economic Entomology 101: 365-374.

Vernon, R.S., van Herk, W., Clodius, M., and C. Harding. 2009. Wireworm management I: Stand protection versus wireworm mortality with wheat seed treatments. Journal of Economic Entomology 102: 2126-2136.