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Section L: Laboratory Bioassays

OXFORD

MOOSE: *Alces alces* (L.)

Pathogenicity of *Metarhizium anisopliae* and *Metarhizium brunneum* Isolates and Efficacy of Met52 G Against Winter Tick Larvae, 2019

Cheryl Frank Sullivan,[1,3] Bruce L. Parker,[1] Agrin Davari,[1] Mi Rong Lee,[2] Jae Su Kim,[2] and Margaret Skinner[1]

[1]Entomology Research Laboratory, University of Vermont, Burlington, VT, [2]Department of Agricultural Biology, Jeonbuk National University, Jeonju, Republic of Korea, Phone: (802)-656-5434 (C.F.S.), (802)-656-5440 (B.L.P., A.D., M.S.), (+82)-63-270-2525 (M.R.L., J.S.K.); Fax: (802)-656-5441 (C.F.S.), (802)-656-5441 (B.L.P., A.D., M.S.), (+82)-63-270-2531 (M.R.L., J.S.K.) (cfrank@uvm.edu; bparker@uvm.edu; adavari@uvm.edu; mimilee248@naver.com; jskim10@jbnu.ac.kr; mskinner@uvm.edu) and [3]Corresponding author, e-mail: cfrank@uvm.edu

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Moose | *Alces alces*

Winter tick | *Dermacentor albipictus* (Packard)

The winter tick, *Dermacentor albipictus* (Packard), is a one-host tick that infests large ungulates and causes significant mortality in moose, *Alces alces* (L.). The off-host, larval stage aggregates on the ground in a quiescent state during summer until they quest for hosts on foliage in autumn. This allows an opportunity to treat a vulnerable stage prior to host recruitment. The objectives were 1) evaluate the pathogenicity of the biological control agents *Metarhizium brunneum* (Petch) strain F52 and two experimental *Metarhizium anisopliae* (Metschn.) Sorokin isolates within the application rates of commercial products, 2) evaluate the efficacy of Met52 G (Novozymes Biologicals, Inc.), containing the AI *M. brunneum* F52, and 3) compare Met52 G to Met52 EC under simulated field conditions.

Larvae were reared from eggs that originated from adult females collected from a deceased moose calf in northeastern, VT, United States. Fungal isolate *M. brunneum* F52 originated from Met52 G and experimental *M. anisopliae* isolates ERL1582 and JEF290 from forest soils in VT, United States and South Korea, respectively. Fungal isolates were cultured for 14 d at ±25°C on potato dextrose agar. Conidial suspensions were prepared in a solution of 0.02% Silwet L-77 (Helena Agri-Enterprises, LLC) and sterile distilled water (SDW). Three concentrations (1×10^6, 1×10^7, and 1×10^8 conidia/ml) for each isolate and two checks (0.02% Silwet + SDW solution [base check] and SDW only [untreated check]) were tested. Ten, 12-wk-old larvae were treated using an immersion method by shaking with 1 ml of suspension in a 1.5 ml microcentrifuge tube for 1 min. Ticks were poured onto filter paper then transferred via. paintbrush to a 50 × 9 mm, tight-fit lid Petri dish lined with 47 mm filter paper pre-moistened with 250 µl SDW. Dishes were placed in a white, plastic seed germination tray with a 2-oz cup with water and covered with a clear humidity dome to maintain RH >90%. Trays were and held at ± 25°C on a benchtop with a photoperiod of 15:9 (L:D) h. Each treatment was replicated three times and mortality was assessed every 3 d for 3 wk.

Met52 G contained 9×10^8 conidia/g and the recommended rate for broadcast applications to turf for ticks is 0.45 kg (low) - 1.36 kg (high)/92.9 m^2. High, medium, low rates, and an untreated check were tested. The amount applied per 0.002 m^2 Petri dish was 27 mg (2 granules), 18 mg (1 granule), and 9 mg (1/2 granule). Ten, 10-wk-old larvae were added to the center of a tight-fit lid Petri dish with SDW moistened filter paper. Granules were dropped into the dish at 10 cm ht. Dishes were placed in trays and held as previously described. Mortality was assessed weekly for 3 wk. Each treatment had five replicates and the experiment repeated three times over three consecutive days.

Enclosures simulating natural conditions were constructed from 34-oz polypropylene containers with lids retrofitted with fine mesh. Each contained a vertical, nylon rod for questing, a base layer of stone, sand, 50–70 ml SDW and 0.037 g ~ 500 eggs within 2-wk of eclosion. Larvae were treated either in summer during quiescence or when active and questing in autumn. High label rates of Met52 G andMet52 EC and an untreated check were tested. Met52 G was applied at 120 mg/0.007 m^2 enclosure. Met52 EC contained 2×10^9 conidia/g with an application rate of 88.72 ml/92.9 m^2 for foliar turf applications. A solution was prepared in SDW where 1.21 ml was sprayed using a hand-held, finger-tip sprayer affixed to a 15 ml conical centrifuge tube. Every 2–4 wk larval mortality was assessed using a percent mortality rating: 0 = no mortality; 1 = 1–25%; 2 = 26–50%; 3 = 51–75%; 4 = 76–99%; and 5 = 100%. Treatments were replicated four times in the summer and five in the fall applications. For all trials, spore viability was determined and adjusted to reflect viability >95%.

Data on the percent mortality and mortality ratings were analyzed using a general linear model with repeated measures and univariate procedures. Analyses for the isolate and granular experiments were followed by Tukey's honestly significant difference (HSD) and for the enclosure experiment, Fisher's least significant difference (LSD). Analyses were conducted using SPSS ver.26 (IBM Corp.) at the $\alpha = 0.05$ level of significance.

The three *Metarhizium* spp. isolates tested were pathogenic to winter tick larvae. Significant differences in percent mortality were observed within each fungal isolate treatment when compared with the checks over 21 DAT (Table 1). Over 50% mortality was observed within 12 DAT for all isolates at concentrations of 1×10^7 and 1×10^8 conidia/ml where isolate Met52 reaching that mortality level within 6 DAT. Met52 and JEF290 showed a significant concentration-dependent virulence over time: Met52 ($F_{12,36}$ = 10.49; $P < 0.001$), JEF290 ($F_{12,36}$ = 3.36; $P = 0.002$), ERL1582 ($F_{12,36}$ = 1.32; $P = 0.252$).

Significant differences in percent mortality were observed among the Met52 G product treatment rates when compared with the check throughout 21 DAT ($F_{6,96}$ = 10.62; $P < 0.001$) (Table 2). Mortality ranged from 72% (low rate) to 89% (high rate) 21 DAT. No significant differences were observed when comparisons were made among the three fungal application rates during the experiment duration ($F_{4,72}$ = 0.75; $P = 0.559$). In general, mortality significantly increased over time ($F_{2,72}$ = 89.52; $P < 0.001$) regardless of fungal application rates ($F_{2,36}$ = 3.13; $P = 0.056$).

In enclosures simulating natural conditions, significant differences in the mortality index were observed among the treatments when Met52 applications were made during the summer from larval quiescence through questing ($F_{14,63}$ = 6.60; $P < 0.001$) (Tables 3 and 4). By 10

Table 1.

Treatment/formulation	Rate (conidia/ml)	Cumulative % mortality						
		3 DAT	6 DAT	9 DAT	12 DAT	15 DAT	18 DAT	21 DAT
Untreated check	-	0.0a	0.0c	0.0c	0.0d	0.0e	0.0c	0.0e
Base check	-	0.0a	0.0c	0.0c	0.0d	0.0e	0.0c	0.0e
Met52	1×10^6	0.0a	10.0bc	23.3bc	30.0bcd	40.0bcde	46.7abc	46.7bcde
Met52	1×10^7	0.0a	76.7a	96.7a	96.7a	96.7a	96.7a	100.0a
Met52	1×10^8	6.7a	50.0ab	96.7a	100.0a	100.0a	100.0a	100.0a
JEF290	1×10^6	0.0a	6.7bc	6.7c	10.0cd	13.3de	26.7bc	30.0de
JEF290	1×10^7	3.3a	10.0bc	43.3abc	56.7abc	56.7abcd	60.0ab	60.0abcd
JEF290	1×10^8	6.7a	40.0abc	73.3ab	83.3a	83.3ab	96.7a	96.7ab
ERL1582	1×10^6	0.0a	3.3c	20.0bc	23.3bcd	26.7cde	40.0bc	43.3cde
ERL1582	1×10^7	0.0a	26.7bc	50.0abc	66.7ab	73.3abc	76.7ab	83.3abc
ERL1582	1×10^8	0.0a	26.7bc	50.0abc	60.0abc	80.0ab	80.0ab	83.3abc
$F_{10,22}$		2.47	7.61	10.22	13.21	15.65	12.03	13.11
P		0.037	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means within a column followed by the same letter are not significantly different ($P > 0.05$, Tukey's HSD).

Table 2.

Treatment/formulation	Rate[a] (conidia/Petri dish)	Cumulative % mortality		
		7 DAT	14 DAT	21 DAT
Untreated Check	-	0.0b	0.0b	0.0b
Met52 G	1×10^7	29.3a	57.3a	72.0a
Met52 G	2×10^7	35.3a	61.3a	76.0a
Met52 G	4×10^7	38.0a	76.7a	89.3a
$F_{3,48}$		20.94	41.07	59.02
P		<0.001	<0.001	<0.001

Means within a column followed by the same letter are not significantly different ($P > 0.05$, Tukey's HSD).
[a]Amount per 0.002 m^2 Petri dish.

Table 3.

Treatment/formulation	Rate[a] (conidia/enclosure)	Cumulative mortality rating[c]							
		3 WAT[b]	6 WAT	8 WAT	10 WAT	14 WAT	18 WAT	21 WAT	23 WAT
Untreated check	-	0.0	0.0	0.0a	0.0a	0.0a	0.8a	1.5a	3.3a
Met52 EC	2.4×10^7	0.0	0.0	0.3a	0.3a	2.3b	2.5a	4.0b	5.0b
Met52 G	1.0×10^8	0.0	0.0	0.5a	1.5b	2.5b	4.8b	4.8b	5.0b
$F_{2,9}$		-	-	1.29	13.29	4.33	11.58	27.80	13.36
P		-	-	0.323	0.002	0.048	0.003	<0.001	0.002

Means within a column followed by the same letter are not significantly different ($P > 0.05$, Fisher's LSD). Products were applied on 14 Jun, 23 Aug. Experiment duration was Jun–Nov.
[a]Amount applied per 0.007 m^2 enclosure.
[b]WAT = weeks after initial treatment.
[c]Percent mortality rating: 0 = no mortality; 1 = 1–25%; 2 = 26–50%; 3 = 51–75%; 4 = 76–99%; and 5 = 100%.

Table 4.

Treatment/formulation	Rate[a] (conidia/enclosure)	Cumulative % mortality[c]							
		3 WAT[b]	6 WAT	8 WAT	10 WAT	14 WAT	18 WAT	21 WAT	23 WAT
Untreated check	-	0.0	0.0	0.0a	0.0a	0.0a	0.8a	13.5a	58.5a
Met52 EC	2.4×10^7	0.0	0.0	0.3a	0.3a	33.5b	38.5a	76.0b	100.0b
Met52 G	1.0×10^8	0.0	0.0	0.5a	13.5b	38.5b	95.2b	95.2b	100.0b
$F_{2,9}$		-	-	1.29	13.29	4.33	11.58	27.80	13.36
P		-	-	0.323	0.002	0.048	0.003	<0.001	0.002

Means within a column followed by the same letter are not significantly different when analyses were conducted on mortality ratings ($P > 0.05$, Fisher's LSD). Products were applied on 14 Jun, 23 Aug. Experiment duration was Jun–Nov.

[a]Amount applied per 0.007 m^2 enclosure.

[b]WAT = weeks after initial treatment.

[c]Transformed from the mean mortality rating.

Table 5.

Treatment/formulation	Rate[a] (conidia/enclosure)	Cumulative mortality rating[c]			
		3 WAT[b]	6 WAT	9 WAT	11 WAT
Untreated check	-	1.6a	2.4a	3.4a	4.2a
Met52 EC	2.4×10^7	3.8b	4.4a	4.8a	5.0b
Met52 G	1.0×10^8	1.0a	2.8a	3.6a	5.0b
$F_{2,12}$		7.24	2.67	2.61	4.57
P		0.009	0.110	0.115	0.033

Means within a column followed by the same letter are not significantly different ($P > 0.05$, Fisher's LSD). Experiment duration was Sept–Dec. Products were applied on 9 Sept.

[a]Amount per 0.007 m^2 enclosure.

[b]WAT = weeks after treatment.

[c]Percent mortality rating: 0 = no mortality; 1 = 1–25%; 2 = 26–50%; 3 = 51–75%; 4 = 76–99%; and 5 = 100%.

Table 6.

Treatment/formulation	Rate[a] (conidia/enclosure)	Cumulative % mortality[c]			
		3 WAT[b]	6 WAT	9 WAT	11 WAT
Untreated check	-	16.0a	36.0a	61.0a	80.8a
Met52 EC	2.4×10^7	71.0b	85.6a	95.2a	100.0b
Met52 G	1.0×10^8	1.0a	46.0a	66.0a	100.0b
$F_{2,12}$		7.24	2.67	2.61	4.57
P		0.009	0.110	0.115	0.033

Means within a column followed by the same letter are not significantly different when analyses were conducted on mortality ratings ($P > 0.05$, Fisher's LSD). Experiment duration was Sept–Dec. Products were applied on 9 Sept.

[a]Amount per 0.007 m^2 enclosure.

[b]WAT = weeks after treatment.

[c]Transformed from the mean mortality rating.

WAT (Aug), mortality in Met52 G was significantly greater than Met52 EC and the untreated check. No check mortality was observed until 18 WAT (mid-Oct, larval age ~4.5 mo) where Met52 G had significantly higher mortality than in Met52 EC and check treatments. When active, questing-age larvae were treated, significant differences in percent mortality were observed among the treatments over the experiment duration ($F_{6,36} = 3.18$; $P = 0.013$) (Tables 5 and 6). No mortality was observed at the time of treatment application, and by 3 WAT (Oct), mortality in the Met52 EC treatment was significantly greater than Met52 G and untreated check treatments. Differences between treatments were not significant for the remainder of the experiment where at 11 WAT (Dec), live ticks were observed only in the untreated check with 100% mortality observed in the Met52 G and Met52 EC treatments. While 100% mortality was observed in the simulated field enclosures, this was 23 WAT (summer) and 11 WAT (fall) and something unlikely to observe in a natural environment. These results confirmed winter tick larvae are susceptible to formulations of *M. brunneum* F52 and demonstrate the use of a granular applied prior to their autumn questing phase.[1]

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