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myo-Inositol and Phytate Are Toxic to Formosan Subterranean Termites (Isoptera: Rhinotermitidae)

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ABSTRACT Several rare and common monosaccharides were screened for toxic effects on the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, with the aim of identifying environmentally friendly termiticides. *myo*-Inositol and phytic acid, which are nontoxic to mammals, were identified as potential termite control compounds. Feeding bioassays with termite workers, where both compounds were supplied on filter paper in concentrations from 160.2 to 1,281.7 $\mu\text{g}/\text{mm}^3$, showed concentration-dependent toxicity within 2 wk. Interestingly *myo*-inositol was nontoxic when administered to termites in agar (40 mg/ml) in the absence of a cellulosic food source, an unexplained phenomenon. In addition, decreased populations of termite hindgut protozoa were observed upon feeding on *myo*-inositol but not phytate-spiked filter paper. Radiotracer feeding studies using *myo*-inositol-[2-³H] with worker termites showed no metabolism after ingestion over a 2-d feeding period, ruling out metabolites responsible for the selective toxicity.

KEY WORDS *Coptotermes formosanus*, carbohydrate, sugar, termite, *myo*-inositol

Of the 28 species in the genus, the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is the most destructive and economically important worldwide (Hardy 1988, Henderson 2001, Woodson et al. 2001, Messenger et al. 2002, Su 2003). *C. formosanus* infestations damage wooden structures of >50 species of living plants (Grace et al. 1996, Osbrink et al. 1999, Messenger et al. 2000, Lax and Osbrink 2003), and damage noncellulosic materials such as insulation on buried electrical and telephone wires (Henderson and Dunaway 1999). The first general survey of an exotic *C. formosanus* infested region in the continental United States was conducted in Louisiana in 1966 (Spink 1967), and the U.S. populations are growing rapidly (Woodson et al. 2001, Lax and Osbrink 2003). A New Orleans, LA, study found termite alates increased by a factor of 14 over a 7-yr period (Henderson 1996).

C. formosanus digest their wood diets using exogenous endo- β -1,4-glucanase (cellulase) and β -glucosidase (cellobiase) enzymes secreted into phagocytotic lumens by symbiotic hindgut protozoa, which are cru-

cial in cellulose and lignin metabolism (Yoshimura et al. 1993a,b; Itakura et al. 1997; Watanabe et al. 2002). They also have endogenous endo- β -1,4-glucanase and β -glucosidase enzymes expressed in the salivary glands and midgut (Itakura et al. 1997, Nakashima et al. 2002, Zhu et al. 2005). *Pseudotrichonympha grassii* Koidzumi, *Holomastigotoides hartmanni* Koidzumi, and *Spirotrichonympha leidyi* Koidzumi comprise the obligatory *C. formosanus* hindgut protozoan population (Yoshimura 1995), which themselves have obligatory intracellular bacterial symbionts (Noda et al. 2005, 2007). Coexisting with these organisms are numerous species of bacteria involved in methanogenesis, acetogenesis, nitrogen fixation, sulfate reduction, and oxygen scavenging (Mertins et al. 1973, Leadbetter and Breznak 1996, Schmitt and Brune 1999, Tholen and Brune 1999, Boga and Brune 2003, Adams and Boopathy 2005) that combine to form a complex and remarkable ancient microecosystem.

Our interest in investigating carbohydrates as termiticides was originally sparked by reports that *D*-tagatose, a rare sugar, was toxic to fly maggots, and was commercialized as Flycracker by Biospherics Inc. (Rockville, MD; Levin and Zehner 1992, Spherix 2002), and is now marketed as an artificial low-calorie sweetener. We screened a number of rare sugars including *D*-tagatose and 2-deoxy-*D*-galactose. While *D*-tagatose had no effect on termites in toxicity screening experiments, 2-deoxy-*D*-galactose was found to be a potent termiticide, causing significant termite mortality and decreasing hindgut protozoan populations (Veillon et al. 2010). Among the library of sugars tested, *myo*-inositol (Figs. 4, 6, 8, and 13) and

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Fig. 1. Chemical structure of *myo*-inositol.

Fig. 3. Chemical structure of phytate.

phytate (Fig. 6) were initially used as controls and were unexpectedly toxic.

Of the nine isomeric forms of cyclohexanehexol, *myo*-inositol is the isomer most common in biology, although at least five others are found (*chiro*-, *epi*-, *muco*-, *neo*-, and *scyllo*-inositols). Unfortunately, for this study, the latter stereoisomers were not available in sufficient quantities to test. *myo*-Inositol, sold as a dietary supplement in the United States, is biosynthesized *de novo* from glucose-6-phosphate in a two-step enzymatic process. First, a *myo*-inositol-3-phosphate synthase-catalyzed NAD^+ -dependent reaction cyclizes glucose-6-phosphate to *D*-*myo*-inositol-3-phosphate (Donahue and Henry 1981; Chen et al. 2000; Loewus and Murthy 2000; Majumder et al. 2003; Daiyasu et al. 2005; Stieglitz et al. 2005), and second, inositol monophosphatase catalyzes dephosphorylation (Stieglitz et al. 2007).

Of the many phosphorylated *myo*-inositol derivatives, phytate, also known as phytic acid and *myo*-inositol-1,2,3,4,5,6-hexakisphosphate, is the most abundant. It is accumulated in plant organs and tissues including tubers, turions, roots, and pollen, and is the principal form of phosphorus storage in seeds (Cosgrove 1980; Raboy 1997). The clearest function is for storing and retrieving phosphorus, chelated calcium, other metals, and *myo*-inositol throughout development and germination. Phytate is ubiquitous in eukaryotes and

usually the most plentiful inositol derivative in cells (Sasakawa et al. 1995), where it and other phosphorylated *myo*-inositol derivatives are involved in numerous functions outside nutrient storage.

myo-Inositol and phytate exhibited concentration-dependent toxicity on *C. formosanus*. Inositol also had toxic effects on *C. formosanus* hindgut symbionts.

Fig. 2. Chemical structure of *myo*-inositol-[2- ^3H].

Fig. 4. Means ($\pm$ SE) of *C. formosanus* percent mortality following treatment of food source filter paper with *myo*-inositol in no-choice assays with 20 workers ($n = 3$). Collection group C was used and the data were recorded daily for 14 d. Means followed by the same letter are not significantly different ($P > 0.05$). The F value for percent mortality is 212.68. P and df values are <0.0001 and 74, 150, respectively. (Online figure in color.)

Fig. 5. Means ($\pm$ SE) of *C. formosanus* filter paper consumption per surviving termite over 14 d. Food source filter paper was treated with *myo*-inositol before incubation. Collection group C survival data were recorded daily for 2 wk. Consumption data were recorded at the end of the 2-wk assay. Groups with statistically similar total consumption values bear the same letter ($P > 0.05$). The total consumption F and P values are 45.99 and 0.0001, respectively. The df values are 3 and 8. (Online figure in color.)

Materials and Methods

Termite Collection and Colony Maintenance. Worker *C. formosanus* were collected from Brechtel Park, New Orleans, LA, according to methods described by Smith et al. (2004). Open-mesh plastic containers filled with a lattice of southern yellow pine or spruce-pine-fir sapwood were buried near a *C. formosanus*-infested tree and recovered after 3–9 wk. Termite-containing crates were held in 250-liter plastic garbage cans kept at room temperature (26–28°C) and 70–80% relative humidity with the original food source. Collections were as follows: 1 March 2008 (group A, *myo*-inositol radiotracer study); 9 May 2008 (group B, *myo*-inositol and *D*-glucose artificial agar diet-termite assay); 29 May 2008 (group C, *myo*-inositol 1,281.7, 640.8, and 320.4 µg/mm³ dose-mortality assay, and *myo*-inositol and phytate 1,281.7, 640.8, and 320.4 µg/mm³ dose-mortality assay); 20 March 2009 (group D, *myo*-inositol, 2-deoxy-*D*-galactose, *D*-glucose and *D*-galactose glucose artificial agar diet-termite assay); 29 May 2009 (group E, *myo*-inositol 1,281.7, 640.8, 320.4 and 160.2 µg/mm³ dose-mortality assay); and 11 May 2010 (group F, both *myo*-inositol and phytate protozoa quantitation). Termites were extracted on moistened filter paper after tapping infested wood sticks into clean plastic containers.

Chemicals. Neutral red dye, *myo*-inositol, phytate, *myo*-inositol-[2-³H], 2-deoxy-*D*-galactose, and *D*-sucrose were obtained from Sigma-Aldrich (St. Louis, MO). *D*-galactose and *D*-glucose were obtained from Matheson Coleman and Bell (Cincinnati, OH) and Fisher (Fair Lawn, NJ), respectively. Structures of *myo*-inositol, *myo*-inositol-[2-³H], and phytate are presented in Figs. 1–3, respectively.

Dose-Response Feeding Assays. Test compounds were applied to 42.5-mm filter papers, in 60- by 15-mm

Fig. 6. Means ($\pm$ SE) of *C. formosanus* percent mortality following treatment of food source filter paper with *myo*-inositol or phytate in no-choice assays with 20 workers ($n = 3$). Collection group C was used and the data were recorded daily for 14 d. Means followed by the same letter are not significantly different ($P > 0.05$). The F value for percent mortality is 25.76. P and df values are <0.0001 and 119, 240, respectively. (Online figure in color.)

plastic petri dishes. One milligram of carbohydrate per 10 µl of distilled water (dH₂O) was the stock solution, using 2.5 mg (160.2 µg/mm³ of filter paper), 5 mg (320.4 µg/mm³), 10 mg (640.8 µg/mm³), and 20 mg (1,281.7 µg/mm³) of *myo*-inositol. Phytate was tested with the three higher concentrations. dH₂O was added to bring the volume to 200 µl and all control filter papers received 200 µl of dH₂O. To prevent desiccation, 100 µl of dH₂O was applied to filter papers throughout the trials pro re nata, approximately every third day. Twenty worker termites were acclimated in the dark in a Parafilm-sealed petri dish at room temperature for 2 wk. Termite mortality was recorded daily for 14 d in triplicate experiments. A one-way analysis of variance (ANOVA), performed using SAS/STAT software (version 9.1), followed by Tukey's studentized range test was used to evaluate statistical differences among groups (SAS Institute 2002, Cary, NC). All mortality data were judged at $\alpha = 0.05$.

Fig. 7. Means ($\pm$ SE) of *C. formosanus* filter paper consumption per surviving termite over 14 d. Food source filter paper was treated with *myo*-inositol or phytate before incubation. Collection group C survival data were recorded daily for 2 wk. Consumption data were recorded at the end of the 2-wk assay. Groups with statistically similar total consumption values bear the same letter ($P > 0.05$). The total consumption F and P values are 38.15 and 0.0001, respectively. The df values are 6 and 14. (Online figure in color.)

Table 1. Parameters used in evaluating CLL modeling of time-concentration-mortality for *C. formosanus* workers subjected to *myo*-inositol and phytate

Treatment	df	Scaled deviance	Mean deviance	Slope (β)	SE of B
<i>myo</i> -Inositol	533	1358.4	2.59	0.9297	0.0842
Phytate	178	355.16	2	1.46	0.3413

Termites from collection group C were used in both the *myo*-inositol 1,281.7, 640.8, and 320.4 µg/mm³ dose-mortality assay and the *myo*-inositol and phytate 1,281.7, 640.8, and 320.4 µg/mm³ dose-mortality assay, and termites from collection group E were used in the *myo*-inositol 1,281.7, 640.8, 320.4, and 160.2 µg/mm³ dose-mortality assay.

In paper consumption experiments, filter papers were weighed before sugar application. After 14 d, termites were removed from the dishes and filter paper from each replicate was cleaned, washed of residual carbohydrate, and dried at 100°C for 24 h. After drying and acclimated for 24 h, filter papers were reweighed. Statistical differences among groups were evaluated using ANOVA followed by Tukey's Student-

Fig. 8. Means ($\pm$ SE) of *C. formosanus* percent mortality following treatment of food source filter paper with *myo*-inositol in no-choice assays with 20 workers ($n = 3$). Collection group E was used and the data were recorded daily for 14 d. Means followed by the same letter are not significantly different ($P > 0.05$). The F value for percent mortality is 134.81. P and df values are <0.0001 and 74, 150, respectively. (Online figure in color.)

Fig. 9. Relationship between observed and CLL model predicted percentage mortality, *myo*-inositol concentration, and time for *C. formosanus* workers. (Online figure in color.)

tized range test. All consumption data were judged at $\alpha = 0.05$.

Complementary Log-Log (CLL) Modeling. A serial time–concentration–mortality design was implemented, and conditional mortality probability was estimated using the CLL model described by Robertson and Preisler (1992). SAS PROC GENMOD (SAS Institute 2002) was used to obtain maximum likelihood estimates of the conditional response parameters, which were in turn used to estimate cumulative mortality probabilities (Robertson and Preisler 1992). A small positive amount was added to all concentration levels to include controls (Robertson and Preisler 1992). LC_{50} and LC_{90} values with confidence limits were estimated for *myo*-inositol and phytate using the formulae given by Robertson and Preisler (1992), and LT_{50} values were estimated by linear interpolation (Nowierski et al. 1996, Feng et al. 1998). In addition, pooled *myo*-inositol data were subjected to ANOVA followed by Tukey's Studentized range test. All data were judged at $\alpha = 0.05$.

Protozoan Counting. The effect of *myo*-inositol and phytate on *C. formosanus* hindgut protozoan populations was examined as follows: Twenty milligrams of *myo*-inositol or phytate were dissolved in 200 μ l of dH_2O and applied to filter paper, in a 60- by 15-mm plastic petri dish ($1,281.7 \mu g/mm^3$). Control filter pa-

Fig. 10. Relationship between observed and CLL model predicted percentage mortality, phytate concentration, and time for *C. formosanus* workers.

per received 200 μ l of dH_2O . Seventy-five worker termites were placed in each dish and collection group F was used in both *myo*-inositol assays and the phytate assay. *P. grassii*, *H. hartmanni*, and *S. leidyi* Koidzumi were counted daily for 2 wk, as described by Manesmann (1972) and modified by Maistrello et al. (2002). Hindguts were removed from three workers and gently macerated in 40 μ l of saline solution containing neutral red dye (0.5 ml of 1% aqueous neutral solution dissolved in 10 ml of saline solution). The number of each protozoan species was determined with a hemocytometer (Bright-line Improved Neubauer, Hausser Scientific, Horsham, PA) under a light microscope. The population of each protozoan species per hindgut (X_F) was calculated as: $X_F = (G \times n) / (V \times 3)$, where G = volume (μ l) of solution in which hindguts were dissected; n = mean of two counts within hemocytometer; and V = volume (μ l) of area counted. Mean ($\pm$ SE) X_F values calculated from two replicates were used for graphical comparison of data. Protozoan population data were subjected to ANOVA followed by a Tukey's Studentized range test, all data were judged at $\alpha = 0.05$. A square-root transformation was applied for data analysis; however, untransformed means are reported.

***myo*-Inositol Administered in the Absence of Cellulose Feeding Bioassay.** Artificial diets were prepared as described by Tanaka et al. (2006). Briefly, 400 mg/10 ml of selected sugars and 150 mg/10 ml of agar

Table 2. Estimates of LC₅₀ and LC₉₀ in µg/mm³ with 95% confidence intervals at different periods for *C. formosanus* workers

Treatment	Time (d)	LC ₅₀ (95% limits)	Time (d)	LC ₉₀ (95% limits)
<i>myo</i> -Inositol	1	1892807080 ^a ± 2033.5	1	37061322741 ^a ± 2880
	2	1627859.1 ^a ± 93.5	2	31873618.1 ^a ± 114
	3	954130.7 ^a ± 88.15	3	18681960.5 ^a ± 106.35
	4	8778.3 ^a ± 67.3	4	171879.4 ^a ± 73.6
	5	1594.4 ^a ± 65.2	5	31218.1 ^a ± 69
	6	629.9 ± 64.75	6	12334.4 ^a ± 67.3
	7	457.3 ± 64.7	7	8954.1 ^a ± 66.85
	8	335.8 ± 64.65	8	6574.5 ^a ± 66.45
	9	288.4 ± 64.65	9	5647.3 ^a ± 66.25
	10	221.8 ± 64.7	10	4343.1 ^a ± 66
	11	142 ± 64.75	11	2781.3 ^a ± 65.55
	12	116.9 ± 64.85	12	2288 ^a ± 65.4
	13	107.4 ± 64.85	13	2103.9 ^a ± 65.35
	14	88 ± 64.95	14	1723.3 ^a ± 65.2
Phytate	1	CI could not be calculated	1	CI could not be calculated
	2	1450894.6 ^a ± 735.75	2	9691371 ^a ± 1587.2
	3	482701.6 ^a ± 296.25	3	3224245.1 ^a ± 578.45
	4	160184 ^a ± 165.6	4	1069962.4 ^a ± 292.55
	5	65161.5 ^a ± 119.55	5	435251.9 ^a ± 194.75
	6	65161.5 ^a ± 119.55	6	435251.9 ^a ± 194.75
	7	6790.2 ^a ± 74.95	7	45356 ^a ± 99.7
	8	1817 ^a ± 66.9	8	12136.6 ^a ± 79.6
	9	1346.7 ^a ± 66.05	9	8995.7 ^a ± 76.75
	10	705.8 ± 65.05	10	4714.6 ^a ± 71.85
	11	490.4 ± 64.95	11	3275.4 ^a ± 69.7
	12	272.3 ± 65.5	12	1818.9 ^a ± 67
	13	212.5 ± 66.05	13	1419.7 ^a ± 66.15
	14	139.6 ± 67.45	14	932.4 ± 65.2

^a Estimate is extrapolated as the highest concentration used was 1,281.7 µg/mm³.

were dissolved in dH₂O, pH 6.8. Sugar and agar solutions were autoclaved separately. Agar-sugar solutions were poured in 60- by15-mm polystyrene petri dishes 5 mm in depth, allowed to solidify, and then divided into quadrants. *myo*-Inositol, *D*-glucose, 2-deoxy-*D*-galactose, and *D*-galactose were all examined as carbon sources in addition to one group that received unsupplemented agar. In triplicate experiments, 20 worker termites from collection group B, for the *myo*-inositol and *D*-glucose (40 mg/ml agar) assay, or collection group D, for the *myo*-inositol, *D*-glucose, *D*-galactose, 2-deoxy-*D*-galactose (40 mg/ml agar), and agar-alone assay, were placed in 60- by 15-mm poly-

styrene petri dishes and provided with an agar-sugar quadrant to serve as a food and water source. After 14 d, termites were removed from the dishes. Mortality data were subjected to ANOVA followed by Tukey's Studentized range test. All mortality data were judged at α = 0.05.

***myo*-Inositol Radiotracer Study.** Food source filter paper was treated with 25 µl of unlabeled 555 mM *myo*-inositol solution and 10 µl of a 500 nM *myo*-inositol-[2-³H] solution, specific activity 10–20 Ci/mM. Twenty worker termites, from collection group A, were fed treated paper for 2 d and frozen. Frozen termites were homogenized and a Bligh and Dyer

Fig. 11. Concentration-dependent LT₅₀ estimates for *myo*-inositol on *C. formosanus* workers.

Fig. 12. Concentration-dependent LT₅₀ estimates for phytate on *C. formosanus* workers.

Fig. 13. Means ($\pm$ SE) of pooled *C. formosanus* percent mortality following treatment of food source filter paper with *myo*-inositol in no-choice assays with 20 workers. Collection groups C and E were used and the data were recorded daily for 14 d. Means followed by the same letter are not significantly different ($P > 0.05$). For controls, 160.2, 320.4, 640.8, and 1,281.7 $\mu\text{g}/\text{mm}^3$ treatment groups, n values are 18, 3, 9, 9, and 9, respectively. The F value for percent mortality is 115.72. P and df values are <0.0001 and 74, 645, respectively. (Online figure in color.)

(Bligh and Dyer 1959) extraction was performed. Aqueous and lipid fractions were concentrated and liquid scintillation counting (LSC) was used to measure radioactivity. The aqueous soluble fraction was fractionated with a silica column (1 cm in diameter and 8 cm in length) using 4:1 acetonitrile: dH₂O as the mobile phase. Eighty 1-ml fractions were collected and LSC was used to evaluate radioactivity. Radioactive fractions were chromatographed on silica thin-layer plates using 4:1 acetonitrile: dH₂O as the mobile phase. Chromatograms were visualized with iodine vapor and retardation factor (R_f) values were compared with *myo*-inositol standards. Fractions were scraped from the plates with a razor and radioactivity was measured using LSC.

Results

Dose-Response Feeding Assays. *myo*-Inositol (1,281.7, 640.8, and 320.4 $\mu\text{g}/\text{mm}^3$): **Concentration-Dependent Toxicity.** Mortality was significant after 3 d in workers treated with both 1,281.7 and 640.8 $\mu\text{g}/\text{mm}^3$ of *myo*-

inositol compared with controls (Fig. 4). The 640.8 $\mu\text{g}/\text{mm}^3$ treatment induced 100% mortality by day 7 and the 1,281.7 $\mu\text{g}/\text{mm}^3$ treatment by day 5 (Fig. 4). The 320.4 $\mu\text{g}/\text{mm}^3$ treatment did not show significant mortality during the 14-d assay.

myo-Inositol (1,281.7, 640.8, and 320.4 $\mu\text{g}/\text{mm}^3$): **Decreased Paper Consumption.** All three treatments caused a concentration-dependent decrease in filter paper consumption (Fig. 5). During the 2-wk assay, each control worker consumed, on average, 0.162 mg of filter paper (Fig. 5). Individual workers feeding on filter paper treated with *myo*-inositol consumed an average of 0.027, 0.079, and 0.1 mg with the 1,281.7, 640.8, and 320.4 $\mu\text{g}/\text{mm}^3$ treatments; Fig. 5).

myo-Inositol and Phytate (1,281.7, 640.8, and 320.4 $\mu\text{g}/\text{mm}^3$): **Concentration-Dependent Toxicity.** With the 1,281.7 $\mu\text{g}/\text{mm}^3$ treatment, *myo*-inositol induced significant mortality after day 4 (100% on day 6), phytate after day 7 (100% on day 10; Fig. 6). At 640.8 $\mu\text{g}/\text{mm}^3$, *myo*-inositol caused significant mortality after day 6 (100% on day 10), while phytate results were significant after day 12 with 73% mortality by day 14

Fig. 14. Means ($\pm$ SE) of *C. formosanus* hindgut protozoan counts. The hindgut protozoa of workers from collection group F were enumerated for 14 d while termites were allowed to feed on filter paper treated with 1,281.7 $\mu\text{g}/\text{mm}^3$ of *myo*-inositol. Means followed by the same letter are not significantly different ($P > 0.05$). The *F* values for total protozoa, *Pseudotrichonympha*, *Holomastigotoides*, and *Spirotrichonympha* are 21.77 ($P = <0.0001$), 23.12 ($P = <0.0001$), 7.83 ($P = <0.0001$), and 12.18 ($P = <0.0001$), respectively. The df value is 23, 48 for all. (Online figure in color.)

(Fig. 6). At 320.4 $\mu\text{g}/\text{mm}^3$, *myo*-inositol did not cause significant mortality, whereas phytate showed significant mortality after day 11, and 62% mortality after day 14 (Fig. 6).

***myo*-Inositol and Phytate (1,281.7, 640.8, and 320.4 $\mu\text{g}/\text{mm}^3$): Decreased Paper Consumption.** All three concentrations, for both *myo*-inositol and phytate, significantly reduced average cellulose consumption per surviving termite when compared with controls (Fig. 7). Consumption on *myo*-inositol treated filter paper averaged 0.062, 0.05, and 0.116 mg at 1,281.7, 640.8, and 320.4 $\mu\text{g}/\text{mm}^3$ treatments, respectively (Fig. 7). Phytate was more effective than *myo*-inositol at 0.029, 0.045, and 0.064 mg consumption, respectively (Fig. 7).

***myo*-Inositol (1,281.7, 640.8, 320.4, and 160.2 $\mu\text{g}/\text{mm}^3$): Concentration-Dependent Toxicity.** On day 14, mortality was not significant with 160.2 or 320.4 $\mu\text{g}/\text{mm}^3$ *myo*-inositol (Fig. 8), while the 640.8 $\mu\text{g}/\text{mm}^3$ became significant on day 7 (Fig. 8), and the 1,281.7

$\mu\text{g}/\text{mm}^3$ after day 1 with 100% mortality on day 6 (Fig. 8).

Intercollection Group Variation in Dose-Mortality Assays. The effects of 1,281.7, 640.8, and 320.4 $\mu\text{g}/\text{mm}^3$ treatments of *myo*-inositol were examined in three separate mortality bioassays. Collection group E was used in the *myo*-inositol 1,281.7, 640.8, 320.4, and 160.2 $\mu\text{g}/\text{mm}^3$ dose-mortality assay. In the remaining two assays, collection group C was used. We examined variation between groups collected from the field at different times, and also between groups collected from the same holding container at different times (C and C'). Results of ANOVA followed by Tukey's Studentized range test (SAS Institute 2002) indicated no intercollection group variation in resistance to *myo*-inositol induced mortality at the 320.4 $\mu\text{g}/\text{mm}^3$ treatment level. However, at the 640.8 and 1,281.7 $\mu\text{g}/\text{mm}^3$ treatment levels, some variation was observed. Specifically, at the 640.8 $\mu\text{g}/\text{mm}^3$ dose, mortality in group C was significantly different from groups E and C' on

Fig. 15. Means ($\pm$ SE) of *C. formosanus* hindgut protozoan counts. The hindgut protozoa of workers from collection group F were enumerated daily for 14 d while termites were allowed to feed on filter paper treated with 1,281.7 $\mu\text{g}/\text{mm}^3$ of myo-inositol. Means followed by the same letter are not significantly different ($P > 0.05$). The F values for total protozoa, *Pseudotriconympha*, *Holomastigotoides*, and *Spirotrichonympha* are 10.72 ($P = <0.0001$), 26.71 ($P = <0.0001$), 7.76 ($P = <0.0001$), and 3.69 ($P = <0.0001$), respectively. The df value is 29, 60 for all. (Online figure in color.)

days 4–6. At the same dose, mortalities in all three groups were different on day 7, and group E was different from groups C and C' on days 8–10. In addition, at 1,281.7 $\mu\text{g}/\text{mm}^3$, mortality in collection group E was significantly different than both collection groups C and C' on days 2 and 3.

CLL Modeling of Mortality Data. The results of time–concentration–mortality CLL modeling of *C. formosanus* are shown in Table 1. The β parameter from the maximum likelihood estimation in the CLL model is the slope value (β). The relationship between time, concentration, and mortality using the CLL model for myo-inositol and phytate against *C. formosanus* is presented with the observed mortality for comparison in Figs. 9 and 10, respectively. CLL modeling for both compounds resulted in a trend of accumulated mortality over time increasing with concentration. LC_{50} and LC_{90} estimates with 95% CIs for myo-inositol and phytate against *C. formosanus* are shown in Table 2. The highest concentration evaluated for both compounds was 1,281.7 $\mu\text{g}/\text{mm}^3$ (20 mg per 42

mm of filter paper), so concentrations exceeding this value are extrapolated. Figures 11 and 12 depict the LT_{50} estimates for myo-inositol and phytate against *C. formosanus*. Estimated LT_{50} values decreased with increasing concentration (Figs. 11 and 12).

When myo-inositol mortality data were pooled and subjected to ANOVA followed by Tukey's Studentized range test (SAS Institute 2002), mortalities were significantly different from controls after days 3 and 5 in the 1,281.7 and 640.8 $\mu\text{g}/\text{mm}^3$ treatment groups (Fig. 13), but not in the 320.4 and 160.2 $\mu\text{g}/\text{mm}^3$ treatment groups (Fig. 13).

Effect on Protozoan Populations. myo-Inositol Bioassay. There was some variation in results of feeding on filter paper treated with 1,281.7 $\mu\text{g}/\text{mm}^3$ on total protozoan populations, with Test 1 showing significance on day 3 (Fig. 14) for *P. grassii* (Fig. 14) and reduced populations on days 4, 5, and 8, and days 4–8, 11, and 13 for *H. hartmanni* and *S. leidy* (Fig. 14). In Test 2, at 1,281.7 $\mu\text{g}/\text{mm}^3$, myo-inositol-treated protozoan populations became significant on days 8–11

Fig. 16. Means ($\pm$ SE) of *C. formosanus* hindgut protozoan counts. The hindgut protozoa of workers from collection group F were enumerated daily for 8 d while termites were allowed to feed on filter paper treated with $1,281.7 \mu\text{g}/\text{mm}^3$ of phytate. Means followed by the same letter are not significantly different ($P > 0.05$). The F values for total protozoa, *Pseudotriconympha*, *Holomastigotoides*, and *Spirotrichonympha* are 7.49 ($P < 0.0001$), 9.02 ($P < 0.0001$), 5.66 ($P < 0.0001$), and 3.28 ($P = 0.0013$), respectively. The df value is 17, 36 for all. (Online figure in color.)

(Fig. 15) with *P. grassii* populations being significantly lower than controls on days 4, 5, 7, 8, 10, and 11 (Fig. 15). *H. hartmanni* populations were also significantly reduced on days 7, 8, 10, and 11.

Phytate Bioassay on Protozoans ($1,281.7 \mu\text{g}/\text{mm}^3$): No Significant Impact. A $1,281.7 \mu\text{g}/\text{mm}^3$ treatment of phytate did not significantly reduce any of the three protozoan populations (Fig. 16).

Termite Agar Diet Assays. *myo*-Inositol and *D*-glucose (40 mg/ml agar). Reduced Toxicity. Interestingly, in the absence of cellulose, *myo*-inositol failed to induce mortality different from that of *D*-glucose controls (Fig. 17).

***myo*-Inositol, *D*-glucose, *D*-galactose, 2-deoxy-*D*-galactose (40 mg/ml Agar), and Agar Alone. Reduced Toxicity.** Figure 18 shows results with agar-alone, *D*-galactose, and 2-deoxy-*D*-galactose groups. *myo*-Inositol, *D*-galactose, and *D*-glucose diets all failed to induce mortality significantly different from the agar alone group, all resulting

in <9% mortality after 14 d (Fig. 18). As expected from our previous studies (Veillon et al. 2010), and coming into the results here, 2-deoxy-*D*-galactose induced mortality following day 10 and ultimately caused 85% cumulative mortality (Fig. 18).

myo-Inositol-[2- ^3H] Radiotracer Metabolic Study.

The aqueous and lipid soluble layers from a Bligh and Dyer extraction (Bligh and Dyer 1959) of whole-body homogenates from termites fed *myo*-inositol-[2- ^3H], were assayed for radioactivity. A 100- μl sample of the lipid soluble fraction contained low radiolabel and resulted in 40.8 wide counts per minute (CPM) and 17.6 ^3H CPM with 9.9 and 15.08% error, respectively. For comparison, a 100- μl sample of the aqueous layer had 160.9 wide CPM and 131.6 ^3H CPM with 4.99 and 5.51% error, respectively. A blank containing only liquid scintillation fluid resulted in 38.9 wide CPM and 14.4 ^3H CPM with 10.14 and 16.67% error, respectively, not significantly different from the concentrated lipid

Fig. 17. Means ($\pm$ SE) of *C. formosanus* percent mortality following feeding on agar containing 40 mg/ml *myo*-inositol or *D*-glucose in no-choice assays with 20 workers ($n = 3$). Collection group B was used and the data were recorded for 14 d. Means followed by the same letter are not significantly different ($P > 0.05$). The F value for percent mortality is 4.84. P and df values are <0.0001 and 29, 270, respectively. (Online figure in color.)

layer. Radioactivity measured in 80 1-ml fractions collected from chromatography of the aqueous layer on a silica gel column is shown in Fig. 19. The chromatograms show a single peak of radioactivity (Fig. 19), at the R_f of *myo*-inositol on thin layer chromatography (TLC) (Fig. 20). These data support the conclusion that *myo*-inositol is not biochemically changed within 2 d following its consumption by *C. formosanus*.

Discussion

myo-Inositol and phytate are toxic to Formosan subterranean termites, when consuming cellulose. *myo*-Inositol significantly caused mortality when applied to food source filter paper discs at 640.8 and 1,281.7 $\mu\text{g}/\text{mm}^3$ in three independent bioassays (Figs. 4, 6, and 8). Phytate also significantly decreased feeding behavior at concentrations ranging from 320.4 to 1,281.7 $\mu\text{g}/\text{mm}^3$ (Figs. 5–7).

C. formosanus termites have been reported to live in the absence of a food source for at least 12 d and closer to a month without significant mortality (Ibrahim et al. 2004), and in our experiments we have observed survival as long as 40 d. Therefore, with the observation of lower cellulose consumption, starvation does not explain the observed mortality. At the present, we are uncertain if the sugars act as feeding deterrents or if decreased cellulose consumption is a symptom of an overall inositol- or phytate-caused reduction in termite health.

Termites stripped of their protozoan symbionts, by rearing on sugar-supplemented agar diets, experience significant mortality when switched back to cellulosic food sources (Tanaka et al. 2006). Although *myo*-inositol significantly reduces hindgut protozoan populations (Figs. 14 and 15), its toxicity to symbiont populations is a secondary effect. Interestingly, *myo*-

Fig. 18. Means ($\pm$ SE) of *C. formosanus* percent mortality following feeding on agar or agar containing 40 mg/ml *myo*-inositol, *D*-galactose or 2-deoxy-*D*-galactose in no-choice assays with 20 workers ($n = 3$). Collection group days was used and the data were recorded daily for 14 d. Means followed by the same letter are not significantly different ($P > 0.05$). The F value for percent mortality is 28.11. P and df values are <0.0001 and 74, 225, respectively. (Online figure in color.)

inositol is not toxic when it is administered in an agar rather than cellulosic diet (Figs. 17 and 18), whereas 2-deoxy-*D*-galactose maintained its toxicity (Fig. 18).

Fig. 19. Radioactivity detected in unconcentrated and 4 $\times$ concentrated aqueous phase silica column fractions of Bligh and Dyer extract of *C. formosanus* homogenate. Twenty workers from collection group A were used. (Online figure in color.)

Fig. 20. Radioactivity detected in TLC fractions from silica column fractions found to contain tritium plotted against R_f value of *myo*-inositol standard. (Online figure in color.)

Radiotracer studies show *myo*-inositol is not chemically changed following its consumption (Figs. 19 and 20) during the toxicity window, which may imply that it is either not effectively transported across the pre- or hindgut membrane or it is not incorporated in a salvage pathway. Future studies would focus on effects of administration of these compounds on metabolomics and gene expression to pursue the mechanism of action. Meanwhile, it appears that these compounds could be effective and inexpensive termiticides, innocuous to humans, and probably harmless to companion and food and fiber animals and the environment.

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