Effect of Root Extracts of Mexican Marigold, *Tagetes minuta* (Asterales: Asteraceae), on Six Nontarget Aquatic Macroinvertebrates

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ABSTRACT Water-soluble root extracts of the Mexican marigold, *Tagetes minuta* L., composed mainly of the compounds alpha-gurjunene, 5-(But-3-ene-1-inyl)-2,2′-bithiophene, palmitic acid, alpha-terthienyl, and 5-(4-Acetoxy-1-butenyl)-2,2′-bithiophene, were assessed to determine their impact on six species of nontarget aquatic invertebrates. Test organisms were collected from a polluted stream—*Gammarus lacustris* Sars (Amphipoda)—as well as from a local nearly pristine stream—*Lepidostoma pluviale* (Milne) (Trichoptera), *Drunella grandis* (McDunnough) (Ephemeroptera), *Baetis tricaudatis* (Dodds) (Ephemeroptera), *Rhithrogena morrisoni* (Banks) (Ephemeroptera) and *Hydropsyche cockerelli* (Banks) (Trichoptera). Root extract of *T. minuta*, formulated with a commercial combination of petroleum solvents and surfactants, caused mortality to some of the organisms tested. Some mortality was attributable to presence of the inert, surfactant/petroleum-based solvent. For example, with *L. pluviale*, the LT_{50} for 4 ppm marigold root extract formulated with 0.01 ppm inert materials was 54.7 h (CI 48.1, 64.2), and for 0.01 ppm inert materials alone was 69.8 h (CI 55.8, 101.2). Of the macroinvertebrates tested, *R. morrisoni* was the most sensitive to inert materials (LT_{50} 0.01 ppm inert = 4.5 h) and *G. lacustris* was the least sensitive (LT_{50} 0.01 ppm inert = 400 h; LC_{50} 96 h = 1.2 ppm). Marigold root extracts did have a toxic effect at the highest concentration (4 ppm) used on all macroinvertebrates tested. Although previous studies reported the effects of one compound in the root extract, alpha-terthienyl, our research is the first published report evaluating effects of full-formulation marigold root extract on immature aquatic nontarget insect species and a crustacean. If root extracts of *T. minuta* are to be used as a pesticide, further research should focus on effects of inert surfactants used in marigold extract formulation on nontarget macroinvertebrates.

KEY WORDS *Lepidostoma*, *Gammarus*, *Drunella*, *Hydropsyche*, methyl bromide alternatives, plant-based pesticide.

PLANT-DERIVED PRODUCTS are increasingly being used to combat crop pests because they are natural and are often assumed to be safe for the environment. These plant-derived products, however, can have a detrimental effect on the environment.

Extracts of marigolds, *Tagetes minuta* L., *Tagetes erecta* L., and *Tagetes patula* L., can be used as insecticides (Philogene et al. 1985; Perich et al. 1994; Weaver et al.1994, 1997), fungicides (Welty and Prestbye 1993), and nematocides (Miller and Ahrens 1969; Davide 1979). *T. minuta* has insecticidal components in its leaves, flowers, and roots (Wells et al. 1993; Weaver et al. 1994). Marigold roots contain the light-sensitive compound alpha terthienyl, which can suppress nematode populations and improve the growth of plants like tobacco (Miller and Ahrens 1969) and tomatoes (Ijani and Mmbaga 1988).

Whole-plant extracts of *T. minuta* were the most lethal of all three species of *Tagetes* to adults and larvae of the yellow fever mosquito, *Aedes aegypti* (L.) (Perich et al.1994). Larvicidal properties of steam distillates from three *Tagetes* species against the third instar of *A. aegypti* persisted at least 9 d in the aquatic environment (Green et al. 1993). A cercaricidal aqueous infusion of *T. patula* protected snail hosts, *Physa occidentalis*, from the digenetic trematode cercariae, which causes schistosomiasis (Graham et al. 1980). A phototoxin extracted from marigolds, alpha-terthienyl, was shown to be extremely insecticidal against mosquitoes, but did not affect nontarget organisms like the ostracod, caddisfly, and *Physa* sp. (Philogene et al. 1985). However, in a field evaluation of alpha-terthienyl, Dosdall et al.(1991) found that application to stream ecosystems caused catatsrophic drift of several species of nontarget aquatic insects and behavioral changes in crayfish.

If extracts of *T. minuta* were to be used for control of aquatic pests (mosquito larvae) or intermediate hosts of disease-causing organisms (schistosomiasis), or if the plant was used as a pesticidal intercrop, the possibility exists that some marigold compounds may seep into the groundwater and migrate into nearby rivers and streams. Therefore, the effects of marigolds on the environment and nontarget aquatic fauna must be studied in greater detail before they are widely used as an alternative pesticide.

Although previous studies reported differing effects of one compound of the root extract, alpha-terthienyl, our research is the first published report evaluating the

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effects of whole root extracts of *T. minuta* on immature aquatic nontarget insect species and a crustacean. The purpose of our research was to evaluate the effect of insecticidal marigold root extracts on five aquatic insects and one crustacean. We tested the hypothesis that if compounds from the root extract are applied directly to the aquatic ecosystem or if they migrate through the soil into nearby streams and rivers, they will adversely affect and ultimately cause death to nontarget aquatic macroinvertebrates.

Materials and Methods

Selection of Test Species and Collection Sites. Six species of aquatic macroinvertebrates were selected for bioassays April 1997 through June 1998, and were collected from a relatively unpolluted trout stream, Bridger Creek (tributary of the East Gallatin River) (sample site = R6E,T1S, Section 31) and a relatively polluted stream site on the Montana State University campus, Campus Creek. These two creeks had differing communities of invertebrates present. *Gammarus lacustris* Sars (Amphipoda) was collected for the bioassays from Campus Creek but was not present at the East Gallatin site. *Hydropsyche cockerelli* (Banks) (Trichoptera), *Lepidostoma pluviale* (Milne) (Trichoptera), *Drunella grandis* (McDunnough) (Ephemeroptera), *Baetis tricaudatis* (Dodds) (Ephemeroptera), and *Rhithrogena morrisoni* (Banks) (Ephemeroptera) were collected for the bioassay from the East Gallatin site, but these species were rare at the Campus Creek site. Species determinations were made by Dan Gustafson, Department of Biology, Montana State University. These six species were chosen for the following reasons: (1) they were abundant, contributed to lotic communities in coldwater streams in SW Montana, and were widely distributed in such streams in the western United States; (2) they represented different classes and orders; (3) they represented different functional feeding groups; and (4) they had varying sensitivities to environmental (organic) pollution as shown by the Hilsenhoff biotic index (Hilsenhoff 1987).

Plant Extraction. Seeds of *T. minuta* were germinated in a greenhouse in the Plant Growth Center at Montana State University in September 1992. In February 1993 they were potted, three plants per pot. Roots of each plant in each pot were harvested and extracted (in our lab) by simultaneous steam distillation and methylene chloride extraction (Weaver et al. 1994) using a Lickens & Nickerson distillation extractor (Kontes Scientific Glassware and Instrumentation, Vineland, NJ). The resulting extracts were stored under N_2 at −20°C in 7 ml glass vials with a Teflon^R-lined screw cap.

Selection Procedures for Individual Test Organisms. Test organisms were collected from the streams mentioned above on the morning each bioassay was started using a standard D-frame kick net and by shuffle-kicking the stream substrate. Bioassay organisms were selected only if they swam vigorously and in a normal pattern for that species and if they met the size criteria for that species. Size criteria were: *G. lacustris* 0.9–1.2 cm; *L. pluviale* 0.4–0.6 cm (case only); *D. grandis* 0.9–1.2 cm; *B. tricaudatis* 0.4–0.6 cm (minus terminal gills); *R. morrisoni* 0.6–0.9 cm; and *H. cockerelli* 0.8–1.2 cm (without terminal gills). Size criteria were used to maximize the probability of selecting individuals of the same life stage, thereby minimizing response variability because of size. Temperature in the stream was recorded. Macroinvertebrates were then transported to the laboratory in a plastic bucket filled with water directly from the stream. In the laboratory, assay organisms were maintained in cool water under a similar temperature range as the stream where they were collected.

Bioassay Facility–Cold Water Bath Environment. The cold water bath facility for aquatic organisms previously described (Dunkel and Richards 1998) was used for bioassays. Test chambers, 125-ml Erlenmeyer flasks containing 100 ml of partially dechlorinated tap water (pH = 8.2 ± 0.2), were immersed in the 21-liter aquaria. City tap water was sufficiently dechlorinated by passive dissipation for 43 h to a mean (n = 3) total chlorine of 0.05 mg/liter measured colorimetrically (Hach DR/700 Colorimeter, Loveland, CO) with the carboxylate salt of N,N-Diethyl-p-phenylenediamine (Permachem^R Reagents). This chlorine level probably precluded the running of a normal assay with *R. morrisoni.*

Bioassay Procedures. The full formulation of the root extract consisted of two parts, the extract that resulted from the simultaneous steam distillation-methylene chloride extraction and inert ingredients. As the extract was immiscible in water, it was mixed with inert ingredients before creating the water dilution. The inert ingredients were obtained from Agridyne, Salt Lake City, UT, as the inert component of Align^R. The inert ingredients consisted of substances such as surfactants, a stabilizer (mineral oil), and petroleum solvents (Dunkel and Richards 1998). Concentrations of inert ingredients at 8, 4, 2, 1, 0.1, and 0.01 ppm were tested on the organisms initially in the fall of 1997 until we arrived at a relatively nontoxic value of 0.01 ppm. The concentration of the inert ingredients was then maintained at 0.01 ppm for subsequent bioassays in which concentrations ranging from 0.4 ppm to 8 ppm root extract of *T. minuta* were tested. Eight flasks for each treatment including the controls contained four organisms. Test organisms were added to each flask, and behavioral observations were recorded at 0.25, 1, 2, 4, 6, 8, 10, 12, 18, 24, 36, 48, 72, and 96 h after exposure, or until a significant number of organisms had died. Timings were subject to change depending on the invertebrate response. Behavioral observations varied with individual species, but included normal behavior, specific abnormal swimming movements, moribundity, and death. Moribundity was defined as the inability to regain movement of the body after the flask was agitated for 10 s. Death was recorded after two consecutive moribundity readings.

Upon termination of each assay, the test organisms were transferred to 70% ethyl alcohol for long-term preservation. Glassware used in the assay was im-

mersed in 25 g of KOH dissolved in 15 ml water and mixed in 5 liters of isopropyl alcohol for 24 h and then triple-rinsed with hot and cold tap water and deionized water with detergents (Contrad^R or Micron^R).

Measurement of Macroinvertebrate Weight and Head Capsules. Dry weights and head capsules of the macroinvertebrates were measured to determine mean organism size and associated variability. Test organisms were taken from their ethanol storage solution and washed with deionized water to remove the ethanol. They were then dried, one treatment per watch glass, in a drying oven under vacuum, at 50°C, until dry (0.5 h). The organisms were then weighed individually or in groups on an electronic analytical balance (Denver Instruments AB 250 D). After weighing, head capsules were measured using a Leica Wild M3C stereo dissecting scope and an ocular micrometer. The organisms were returned to the ethanol solution for storage after recording measurements for body weight and head capsule width. Different notable landmarks were used for each species. For *G. lacustris*, this measurement was the widest distance between the posterior border of the antennal socket and the posterior border of the photosensitive area. For *L. pluviale*, this measurement was the gap between medial boundaries of the eye sockets. For *D. grandis*, this measurement was the gap between the two projections (tubercles) on the frons. For *R. morrisoni*, this measurement was the narrowest gap between medial ocular borders. For *H. cockerelli*, this measurement was the longest distance between the ventral margin of the frons and the dorsal depression of the vertex.

Analysis of Marigold Root Extract. The GC/MS analysis was performed using a VG 70E-HF double-focusing mass spectrometer. The ion source was operated at 200°C, with an electron current of 100 microamperes and the ion acceleration potential at 6,000 volts. The mass spectra were recorded at a resolution of 1,200, scanning exponentially down over a mass range of 400-45 amu at a scan rate of 0.60 s per mass decade. The Hewlett–Packard 5890 Series II Plus gas chromatograph was operated in the splitless mode. It was equipped with a 30-m HP-5 capillary column (0.25 mm inside diameter, 0.25 micron film thickness). Ultrahigh purity helium (obtained from a local distributer, Bozeman, MT) was used as the carrier gas after having been passed through a water trap, molecular sieve traps, and two oxygen traps (Alltech, Deerfield, IL). Column head pressure was ramped with the oven temperature to maintain near constant and reproducible flow through the column. Initial GC oven temperature and hold time were 30°C and 2 min, respectively. The temperature was increased 5°C per min. to 150°C, followed by a 25°C/min increase to 250°C. Data were acquired for the first 30 min of the analytical separation. The injection port temperature was 260°C and the GC/MS interface temperature was 280°C. The injection volume was 10 microliters via a Hamilton syringe. The GC/MS was controlled by a VG data system using a DEC alpha computer, a VG SIOS interface, and VG software. Compound identification was made by a spectral interpretation, high resolution mass analysis, library searches with the National Institute of Science and Technology (NIST) database (62,235 compounds), and comparison with known authentic materials.

Statistical Analysis. Probit regression analyses (Finney 1952) were calculated on all mortality data (PROC PROBIT, SAS Institute 1988). The LT_{50}, LC_{50}, the slope ± SE, intercept, and formula describing the probit line were obtained. The chi-square probability was determined for each probit line. Statistical analysis included the calculation of probits chi-square values, lethal time for mortality of 50% of the bioassayed organisms (LT_{50}), and lethal concentrations for 50% of the bioassayed individuals (LC_{50}). The body weight and head capsule measurements were statistically analyzed using SAS Institute (1988) to obtain an assay mean and standard deviations within species between assays as well as within assays between treatments. Differences among bioassay means were tested for significance using the Student-Newman-Keuls means comparison test.

Results

Macroinvertebrate Toxicity of Marigold Root Extract. LC_{50}s for the full formulation of marigold root extract ranged from 0.23 to 6.91 ppm at 96 h for five species of aquatic macroinvertebrates. Both the lowest and highest LC_{50}s were obtained for *D. grandis*, with the most sensitivity observed for this species in the spring and the least sensitivity observed in the fall. Other differences noted in these particular bioassays were that individuals in the more sensitive population of *D. grandis* (spring 1998) were 11.4 times larger in dry body weight than those of the fall 1997 population. The size relationship with sensitivity to formulated marigold root extract was more predictable with the two trichopteran species, *L. pluviale* and *H. cockerelli* (Table 1). The smaller trichopteran, *L. pluviale*, was five times more sensitive and 5.7 times smaller than the larger trichopteran, *H. cockerelli.* With *G. lacustris*, only a few test subjects in inert 0.01 ppm and marigold extract at 1 ppm succumbed to the formulation in spring 1998 (and none of the methylene chloride subjects), so it was not possible to run an LC_{50} on the spring 1998 data and obtain a full set of fiducial limits. Repetitions of the LC_{50} determinations within species, within season, were not significantly different (e.g., in the bioassays initiated 8 and 22 September 1997 for *G. lacustris)* (Table 1). Although the concentration of the inert ingredients used in these two assays was different (0.1 ppm on 8 September 1997 and 0.01 on 22 September 1997), the LC_{50} did not show the small differences in toxicity that were apparent in the LT_{50} values (Table 2). For example, the LT_{50} for 4 ppm full formulation (0.1 ppm inert) was 6.5 h compared with 16.2 h for 4 ppm full formulation (0.01 ppm inert). In our preliminary assay, *R. morrisoni* was extremely sensitive. We were therefore not able to obtain LC_{50} data and conducted the assays for this organism with only two treatments (Table 2).

Table 1. Probit analyses of mortality, Pearsons chi-square, LC_{50}, mean weight, and head capsule width using marigold root extract formulated with 0.01 ppm inert material for 96 h against five species of aquatic macroinvertebrates

Species[a]	Assay date	Intercept ± SE	Slope ± SE (Pearsons χ^2 of the slope)	Pearsons χ^2 for goodness-of-fit test	df (no. of covariate values-2)	LC_{50} ppm (95% CI)	Mean wt, g × 10^{-5} ± SD	Mean head capsule width, mm ± SD[b]
G. lacustris	8 Sept. 1997[c]	0.29 ± 0.17	2.83 ± 0.54 (27.56 $P < 0.0001$)	2.61NS	1	1.27 (1.03, 1.66)	—[d]	—[d]
G. lacustris	22 Sept. 1997	0.24 ± 0.17	3.29 ± 0.58 (31.14 $P < 0.0001$)	1.10NS	1	1.19 (1.08, 1.48)	330 ± 20C[b] (n = 25)	0.26 ± 0.005AB[b] (n = 68)
G. lacustris (methylene chloride only)	11 June 1998	−1.59 ± 0.35	0.41 ± 0.22 (3.33 $P < 0.0682$)	0.91NS	1	8072[e]	550 ± 70B (n = 25)	0.29 ± 0.02AB (n = 100)
L. pluviale	16 March 1998	0.16 ± 0.12	0.57 ± 0.20 (8.42 $P < 0.0037$)	0.72NS	2	0.53 (0.14, 1.6)	30 ± 10A[b] (n = 15)	0.25 ± 0.02A[b] (n = 79)
H. cockerelli	27 Oct. 1997	0.37 ± 0.14	0.94 ± 0.34 (7.415 $P < 0.0065$)	0.57NS	1	2.50 (1.3, 37.24)	170 (n = 20)[f]	1.34 (n = 67)[f]
D. grandis	6 Oct. 1997	1.25 ± 0.20	1.49 ± 0.58 (6.46 $P < 0.011$)	1.63NS	1	6.91 (3.11, 1524)	62 ± 20B[b] (n = 12)	0.62 ± 0.023B[b] (n = 41)
D. grandis	28 March 1998	0.46 ± 0.14	0.73 ± 0.34 (4.58 $P < 0.0322$)	0.02NS	1	0.23 (0.61, 1.58)	710 ± 101A[b] (n = 20)	1.20 ± 0.05A[b] (n = 129)
B. tricaudatis (inert material only)	29 Sept. 1997	—[g]	—[g]	—[g]	—[g]	—[g]	—[c]	—[e]

NS, nonsignificant ($P > 0.05$). Chi-square test for goodnss-of-fit. Inert material used = commercial preparation used in Align.

[a] The sixth species, *Rithrogena morrisoni*, was difficult to find in large numbers for a full LC_{50} analysis and also was extremely sensitive to the bioassay system, so it was not possible to obtain analyzable data with serial dilutions to conduct an LC_{50}.

[b] Means compared with a Student-Newman-Keuls test ($P \leq 0.05$) within species between bioassays.

[c] This marigold root extract was formulated with 0.1 ppm inert ingredients.

[d] Body weight/head capsule width could not be determined due to decomposition of stored organisms or specimens not being retained.

[e] Fiducial limits not available for this data set. Methylene chloride is not toxic to *G. lacustris*.

[f] There were no other assays with *H. cockerelli* with which to compare these data.

[g] *Baetis tricaudatis* was extremely sensitive to the inert material used, but not particularly sensitive to the laboratory assay system. At 19 hours when the assay was abandoned, no moribundity or mortality had occurred in the controls, but in the lowest level of inert material used (0.01 ppm), 10 of 30 assay organisms were moribund or dead.

Table 2. Probit analysis of mortality, LT_{50}, using various concentrations of full formulation (containing inert ingredients and marigold extract) or inert ingredients only against 6 species of aquatic macroinvertebrates

Formulation	Assay date	Intercept ± SE	Slope ± SE (Pearsons χ^2 of the slope)	Pearsons χ^2 for goodness-of-fit test	df (no. of covariate values-2)	$LT_{50\,(95\%\ CI)}$ (h)	Mean wt, g × 10^{-5} ± SD	Head capsule width (mm ± SD)[a]
G. lacustris[b]								
Inert 4 ppm	27 Aug. 1997	−8.94 ± 1.31	21.09 ± 2.96 (46.34 $P < 0.0001$)	4.02NS	4	2.65 (2.56, 2.72)	430 ± 200A[c] ($n = 5$)	0.30 ± 0.05A[c] ($n = 7$)
Inert 2 ppm	27 Aug. 1997	−10.0 ± 1.29	14.72 ± 1.85 (62.86 $P < 0.0001$)	3.20NS	4	4.76 (4.6, 4.93)	450 ± 190A[c] ($n = 5$)	0.28 ± 0.05A[c] ($n = 7$)
Inert 1 ppm	27 Aug. 1997	−4.65 ± 0.93	6.53 ± 1.24 (27.46 $P < 0.0001$)	2.21NS	4	5.16 (4.72, 5.52)	500 ± 200A[c] ($n = 5$)	0.27 ± 0.05A[c] ($n = 10$)
Inert 0.4 ppm	27 Aug. 1997	−1.05 ± 0.28	1.00 ± 0.27 (14.12 $P < 0.0002$)	5.63NS	4	11.22 (7.21, 19.4)	370 ± 60A[c] ($n = 5$)	0.27 ± 0.06A[c] ($n = 9$)
Inert 0.1 ppm	1 Sept. 1997	−4.09 ± 0.55	3.75 ± 0.47 (61.54 $P < 0.0001$)	1.5NS	3	11.22 (10.47, 14.13)	—[d]	—[d]
Full 4 ppm (Inert 0.1 ppm)	1 Sept. 1997[e]	−1.62 ± 0.51	0.74 ± 0.33 (4.99 $P < 0.0254$)	0.3NS	2	146.89 (58.88, 102.33)	340 ± 40AB ($n = 5$)	0.24 ± 0.05A ($n = 28$)
Inert 0.1 ppm	8 Sept. 1997	−2.58 ± 0.92	2.25 ± 0.69 (10.49 $P < 0.0012$)	4.7NS	2	14.12[g]	—[d]	—[d]
Full 4 ppm (Inert 0.1 ppm)	8 Sept. 1997	−4.32 ± 1.07	5.29 ± 1.32 (15.92 $P < 0.0001$)	1.3NS	3	6.53 (5.88, 7.34)	—[d]	—[d]
Full 1 ppm (Inert 0.1 ppm)	8 Sept. 1997	−5.39 ± 1.97	2.97 ± 1.07 (7.683 $P < 0.0056$)	0.02NS	1	64.56 (44.66, 81.28)	—[d]	—[d]
Inert 0.01 ppm	8 Sept. 1997	−1.93 ± 2.39	0.47 ± 1.29 (0.13 $P < 0.7192$)	—[f]	0	13,803[g]	—[d]	—[d]
Full 4 ppm (Inert 0.01 ppm)	22 Sept. 1997	−2.31 ± 0.45	1.90 ± 0.46 (17.30 $P < 0.0001$)	11.48	4	16.21 (4.97, 28.8)	330 ± 50A ($n = 5$)	0.25 ± 0.03A ($n = 19$)
Full 1 ppm (inert 0.01 ppm)	22 Sept. 1997	−3.80 ± 1.08	2.00 ± 0.61 (10.11 $P < 0.0015$)	0.45NS	2	85.90 (64.56, 190.54)	320 ± 6A ($n = 5$)	0.25 ± 0.03A ($n = 16$)
Full 0.4 ppm (Inert 0.01 ppm)	22 Sept. 1997	−8.04 ± 5.07	3.64 ± 2.68 (1.83 $P < 0.1754$)	2.866NS	1	158.48[g]	350 ± 60A ($n = 5$)	0.26 ± 0.03A ($n = 16$)
Inert 0.01 ppm	22 Sept. 1997	−8.66 ± 5.08	3.99 ± 2.69 (2.20 $P < 0.1375$)	2.87NS	1	146.55[g]	350 ± 50A ($n = 5$)	0.26 ± 0.02A ($n = 17$)
Inert 0.01 ppm	12 May 1998	−3.72 ± 2.48	1.43 ± 1.33 (1.15 $P < 0.2821$)	—[f]	0	400.02[g]	610 ± 70BC ($n = 5$)	0.26 ± 0.05B ($n = 29$)
Full 1 ppm (Inert 0.01 ppm)	12 May 1998	−2.19 ± 2.03	0.90 ± 1.10 (0.67 $P < 0.4119$)	—[f]	0	267.36[g]	700 ± 50AB ($n = 5$)	—[d]
L. pluviale[b]								
Inert 4 ppm	11 Apr. 1997	−4.30 ± 0.58	12.74 ± 1.61 (62.38 $P < 0.0001$) (42.22 $P < 0.0001$)	2.14NS	4	2.17 (2.08, 2.26)	30 ± 10A ($n = 3$)	0.18 ± 0.03C ($n = 16$)
Inert 2 ppm	11 Apr. 1997	−3.78 ± 0.58	5.73 ± 0.86	1.61NS	5	4.56 (4.20, 4.94)	—[d]	0.24 ± 0.04B ($n = 16$)
Inert 1 ppm	11 Apr. 1997	−2.80 ± 1.09	3.90 ± 1.62 (5.77 $P < 0.0163$)	9.21	3	5.2[b,g]	10 ± 3B ($n = 3$)	0.26 ± 0.03AB ($n = 16$)
Inert 0.4	11 Apr. 1997	−1.28 ± 0.36	0.90 ± 0.38 (7.2 $P < 0.0073$)	8.80NS	4	26.06[b,g]	20 ± 2A ($n = 3$)	0.23 ± 0.04B ($n = 22$)
Full 4 ppm (Inert 1 ppm)	8 May 1997	−2.57 ± 0.48	5.95 ± 0.96 (37.92 $P < 0.0001$)	4.40NS	2	2.70 (2.38, 2.98)	20 ± 3AB ($n = 6$)	0.28 ± 0.03A ($n = 15$)

Table 2. Continued

Formulation	Assay date	Intercept ± SE	Slope ± SE (Pearsons χ^2 of the slope)	Pearsons χ^2 for goodness-of-fit test	df (no. of covariate values-2)	$LT_{50(95\% CI)}$ (h)	Mean wt, $g \times 10^{-5}$ ± SD	Head capsule width (mm ± SD)[a]
Full 0.4 ppm (Inert 1 ppm)	8 May 1997	−1.15 ± 0.20	0.94 ± 0.17 (29.43 $P < 0.0001$)	6.41NS	5	16.21 (10.71, 28.2)	20 ± 4A ($n = 6$)	0.25 ± 0.03AB ($n = 16$)
Full 0.04 ppm (Inert 1 ppm)	8 May 1997	−1.78 ± 0.55	1.25 ± 0.50 (6.23 $P < 0.0125$)	9.02	3	25.94[b,g]	20 ± 3AB ($n = 6$)	0.23 ± 0.03B ($n = 17$)
Inert 0.01 ppm	16 Mar. 1998	−3.82 ± 0.70	2.07 ± 0.42 (24.62 $P < 0.0001$)	2.80NS	4	69.75 (55.77, 101.23)	30 ± 4AB ($n = 3$)	0.28 ± 0.02B ($n = 18$)
Full 4 ppm (Inert 0.01 ppm)	16 Mar. 1998	−5.05 ± 0.76	2.91 ± 0.45 (41.08 $P < 0.0001$)	4.80NS	6	54.72 (48.06, 64.2)	30 ± 11AB ($n = 3$)	0.26 ± 0.02AB ($n = 18$)
Full 0.1 ppm (Inert 0.01 ppm)	16 Mar. 1998	−4.45 ± 0.88	2.32 ± 0.51 (20.46 $P < 0.0001$)	4.72NS	4	83.23 (66.34, 131.52)	43 ± 10A ($n = 3$)	0.23 ± 0.040B ($n = 6$)
Full 0.4 ppm (Inert 0.01 ppm)	16 Mar. 1998	−4.06 ± 1.11	2.01 ± 0.62 (10.47 $P < 0.0012$)	0.50NS	2	106.83 (78.47, 276.75)	—[d]	0.23 ± 0.04B ($n = 22$)
Full 0.1 ppm (Inert 0.01 ppm)	16 Mar. 1998	−3.12 ± 1.16	1.37 ± 0.65 (4.50 $P < 0.0338$)	0.11NS	2	203.19 (104.6, 695.36)	45 ± 9A ($n = 3$)	0.28 ± 0.03A ($n = 15$)
H. cockerelli[b]								
Inert 0.01 ppm	27 Oct. 1997	−3.61 ± 1.58	1.92 ± 0.87 (4.80 $P < 0.0284$)	1.73NS	2	77.27 (58.5, 449.8)	220 ± 70A ($n = 4$)	1.44 ± 0.47A ($n = 10$)
Full 4 ppm (Inert 0.01 ppm)	27 Oct. 1997	−6.03 ± 1.08	3.49 ± 0.62 (32.24 $P < 0.0001$)	0.52NS	4	52.72 (45.92, 59.84)	150 ± 30AB ($n = 4$)	1.36 ± 0.12A ($n = 12$)
Full 1 ppm (Inert 0.01 ppm)	27 Oct. 1997	−6.34 ± 1.57	3.39 ± 0.86 (15.35 $P < 0.0001$)	1.63NS	3	73.96 (64.41, 93.32)	150 ± 20AB ($n = 4$)	1.33 ± 0.07A ($n = 13$)
Full 0.4 ppm (Inert 0.01 ppm)	27 Oct. 1997	−4.41 ± 1.40	2.30 ± 0.78 (8.68 $P < 0.0032$)	0.95NS	3	82.41 (67.0, 157.76)	130 ± 10B ($n = 4$)	1.28 ± 0.13A ($n = 17$)
D. grandis[b]								
Full 4 ppm (Inert 0.01 ppm)	6 Oct. 1997	−4.80 ± 1.25	3.76 ± 0.90 (17.38 $P < 0.0001$)	13.11	4	18.92[g]	—[d]	—[d]
Full 0.4 ppm (Inert 0.01 ppm)	6 Oct. 1997	−9.62 ± 1.19	6.30 ± 0.78 (64.69 $P < 0.0001$)	5.623NS	5	33.41 (31.04, 35.48)	80 ± 20A ($n = 4$)	0.64 ± 0.13A ($n = 13$)
Full 1 ppm (Inert 0.01 ppm)	6 Oct. 1997	−8.88 ± 1.12	5.88 ± 0.74 (63.08 $P < 0.0001$)	3.84NS	4	31.62 (29.65, 35.07)	40 ± 10B ($n = 4$)	0.59 ± 0.09A ($n = 14$)
Inert 0.01 ppm	28 Mar. 1998	−5.55 ± 0.96	3.68 ± 0.62 (35.87 $P < 0.0001$)	2.53NS	2	32.26 (27.07, 37.63)	590 ± 100B ($n = 4$)	1.15 ± 0.14B ($n = 23$)
Full 4 ppm (Inert 0.01 ppm)	28 Mar. 1998	−5.12 ± 1.20	3.45 ± 0.77 (19.84 $P < 0.0001$)	19.09	6	30.45 (20.74, 40.06)	590 ± 140B ($n = 4$)	1.2 ± 0.14AB ($n = 30$)
Full 1 ppm (Inert 0.01 ppm)	28 Mar. 1998	−8.14 ± 0.96	5.24 ± 0.60 (74.08 $P < 0.001$)	7.60NS	5	35.72 (32.65, 38.89)	840 ± 60A ($n = 4$)	1.24 ± 0.13A ($n = 29$)
Full 0.4 ppm (Inert 0.01 ppm)	28 Mar. 1998	−7.91 ± 1.17	4.88 ± 0.72 (46.27 $P < 0.0001$)	1.70NS	4	42.54 (37.75, 45.67)	750 ± 80AB ($n = 4$)	1.18 ± 0.13AB ($n = 27$)

Table 2. Continued

Formulation	Assay date	Intercept ± SE	Slope ± SE (Pearsons χ^2 of the slope)	Pearsons χ^2 for goodness-of-fit test	df (no. of covariate values-2)	$LT_{50(95\% CI)}$ (h)	Mean wt, $g \times 10^{-5} \pm SD$	Head capsule width (mm ± SD)[a]
B. tricaudatis[h]								
Inert 0.01 ppm	29 Sep. 1997	−2.07 ± 1.00	2.07 ± 0.96 (4.62 $P < 0.0316$)	17.50	2	10[d,g]	—[d]	—[d]
Inert 1 ppm	29 Sep. 1997	−5.07 ± 1.29	14.0 ± 3.20 (19.04 $P < 0.0001$)	0.25NS	1	2.30 (2.07, 2.45)	—[d]	—[d]
Inert 0.1 ppm	29 Sep. 1997	−3.33 ± 0.78	6.60 ± 1.31 (25.33 $P < 0.0001$)	2.87NS	2	3.16 (2.71, 3.53)	—[d]	—[d]
Inert 4 ppm	29 Sep. 1997	−9.87 ± 5.59	56.03 ± 31.80 (3.10 $P < 0.999$)	—[f]	0	1.50[g]	—[d]	—[d]
R. morrisoni controls	10 Apr. 1998	−6.38 ± 1.124	7.10 ± 1.31 (29.26 $P < 0.0001$)	1.84NS	4	7.92 (7.38, 8.77)	327 ± 50A ($n = 5$)	0.64 ± 0.20A ($n = 17$)
Inert 0.01 ppm	10 Apr. 1998	−3.13 ± 0.414	4.81 ± 0.59 (64.66 $P < 0.0001$)	5.46NS	6	4.45 (4.06, 4.89)	355 ± 44A ($n = 5$)	0.74 ± 0.24A ($n = 21$)

NS, nonsignificant ($P > 0.05$) chi-square test for goodness-of-fit (df as shown). Invertebrate sizes/weights within species between assays are provided in Table 1; these data within species within assays between treatments are compared in Table 2.

[a] See footnote[b] Table 1. The means comparisons in Table 2 were made within assay, between treatments.

[b] Four invertebrates per flask; 8 flasks per dose.

[c] Mean body weight ($g \times 10^5$)/head capsule (mm) for organisms in the control (no treatment) for this assay were 280 ± 50 A/0.2356 ± 0.0287 A.

[d] Body weight/head capsule width could not be determined due to decomposition of stored organisms or specimens not being retained.

[e] This concentration was prepared 15 h before testing by mixing the *T. minuta* extract with inert ingredients and water and letting stand overnight at room temperature. By morning, the solution was miscible without cloudiness. At 48 h, there were 2 deaths, 1 with 1 ppm full formulation (0.01 ppm inert) and 0.4 ppm full formulation (0.01 ppm inert). No further mortality was observed through the end of test at 96 h.

[f] df = 0 so no Pearsons chi-square could be run with this data.

[g] Fiducial limits not available for this data set.

[h] Five insects per flask; 6 flasks per dose (except 4 ppm full formulation that consisted of 2 flasks).

Development of a formulation began with finding a material that would allow the marigold extract in methylene chloride to mix with the water environment of the macroinvertebrates. We established that methylene chloride at concentrations far exceeding those in our assays would not affect *G. lacustris* (Table 1). We conducted a set of three assays (Table 2; 27 August 1997, 1 September 1998, 8 September 1998) to establish the level of commercial inert ingredients from another plant extract pesticide (Align) that would be effective. The 27 August 1997 assay established that these inert ingredients are strong toxins. In the 1 September 1997 assay, we mixed the marigold root extract and inert material 15 h before testing. The marigold extract at 4 ppm full formulation (0.1 ppm inert) had only a small effect (LT_{50} = 146 h), whereas the 0.1 ppm inert ingredients prepared immediately before testing had a toxic effect (LT_{50} = 11 h). Concentrations used in other studies (4 ppm) with related organisms (Dunkel and Richards 1998) and the four lower concentrations that we used (the lowest being 0.1 ppm) resulted in LT_{50}s <12 h (Table 2) and 100% mortality within the 96 h assay. The 0.01 ppm inert treatment was not toxic to *G. lacustris* in the September 1997 assay, and we proceeded to use this formulation with the root extract to test other macroinvertebrate species. All other species tested had LT_{50}s that were <96 h when placed in an environment with 0.01 ppm inert ingredients (Table 2).

Formulated root extract of *T. minuta* caused mortality to the organisms tested, most of which might be explained by the action of the inert. For example, with *D. grandis*, the addition of root extract of *T. minuta* to the formulation (0.4–4.0 ppm), did not significantly change the LT_{50s} (Table 2).

Chemical Composition of Root Extract of *T. minuta*. Compounds matched with the NIST library at purity of over 900 were: azulene, naphthalene, cyclohexene, 2,2′:5′,2″-terthiophene, heptadecane, hexatriacontane, octadecane, nonadecane, 2,2′-bithiophene, palmitic acid, 2-norpinene, 2-beta-farnesene, benzo(a)phenazine, and pentadecanal.

Macroinvertebrate Response to Assay Environment (Controls). All tested organisms except *R. morrisoni* exhibited no mortality response to the control environment (partially dechlorinated water).

Macroinvertebrate Toxicity of Inert Ingredients in Formulation. Inert ingredients were found to be the most toxic compounds in the formulation. LT_{50}s of the inert concentrations 0.01 ppm to 4 ppm ranged from 1.49 to 42.54 h in three of the six macroinvertebrates tested, *B. tricaudatis*, *R. morrisoni*, and *D. grandis* (all ephemeropterans). *R. morrisoni* was the most sensitive to the extract as well as the inert, and *G. lacustris* was the least sensitive. Time was a factor in the course of moribundity to mortality. Specifically after the initial mortality, the remaining individuals generally progressed more slowly toward moribundity both in the marigold extract formulation and in the inert ingredients alone over time.

The most important result we discovered is that for marigold root extracts to be used in a pesticidal formulation, the least toxic concentration of inert formulation for the extracts to be miscible in water is 0.01 ppm. Also, the marigold root extract at higher concentrations mixed with inert, caused high mortality, and low mortality at lower concentrations.

Body Weight and Head Capsule Measurements of Bioassay Organisms. The means of body weight and head capsules reported in Table 1 can be compared with the grand means of assays reported only in Table 2. For *G. lacustris* body weight ($g \times 10^{-5}$)/head capsule (mm), these grand means were 27 August 1997 assay 410 ± 80C (n = 25)/0.2712 ± 0.0248AB (n = 44); 1 September 1997 assay 340 ± 50C (n = 20)/0.2509 ± 0.0094B (n = 84); 12 May 1998 assay 670 ± 90A (n = 25)/0.3111 ± 0.0615A (n = 86); 3 June 1998 assay 670 ± 80A (n = 30)/0.2847 ± 0.0140AB (n = 118). For *L. pluviale* without case, body weight ($g \times 10^{-5}$)/head capsule (mm), these grand means were 11 April 1997 assay 21 ± 9AB (n = 12)/0.2411 ± 0.0377A (n = 88); 8 May 1997 assay 20 ± 3B (n = 24)/0.2490 ± 0.0195A (n = 71). Sample number varies in the grand means primarily because head capsules did not deteriorate as fast in the ethanol storage system as in macroinvertebrate bodies.

Gammarus lacustris used in the bioassays were significantly larger in the late summer and early fall than those used in the late spring (Tables 1 and 2). However, among the species examined in this study, the only significant difference was between one of the four late summer-early fall bioassays and one of the late-spring bioassays. *D. grandis* used in the bioassays were significantly larger in the spring than those used in the fall (Table 1). *L. pluviale* used in the bioassays were significantly heavier in March 1998 than in May 1997 (Table 1), but their head capsule measurements during this period were not significantly different. When the same analyses were made within bioassays between treatments (Table 2), we found significant differences in body weight between treatments. For example, in the 1 September 1997 bioassay, the *G. lacustris* were significantly larger in the 0.4 ppm *T. minuta* formulation than those in the 1 ppm formulation of *T. minuta.*

Discussion

Seasonal Differences in Sensitivity on *T. minuta*. Seasonal difference in sensitivity of *G. lacustris* to *T. minuta* root extract could be correlated with significant body size differences. For example, *G. lacustris* was more sensitive to inert at 0.01 ppm and 1.0 ppm root extract of *T. minuta* in fall (22 September 1998 bioassay) than in spring (12 May 1998). On these same assay dates, the body weight of these macroinvertebrates was significantly smaller (about one-half the weight) in September than in May (Tables 1 and 2). The endpoint response we measured was death. It is also important to assess levels of moribundity, particularly the earliest indications of moribundity. In our assay system, all observations of moribundity, even the earliest indications, did not result in recovery but led, eventually, to death.

Some chi-square values were significant in assays when the mortality progressed rapidly (Table 2). These data can be interpreted by using a complementary log-log model to model time-dose-mortality relationships from the bioassays (Nowierski et al. 1996). Differing mortality for some organisms might be attributed to the heterogeneity of the field-collected test populations or to the interaction between inert materials and the root extract which might have caused some structural changes in the chemistry of the root extract, thus changing their combined effect within the replicates of the same organism tested. Heterogeneity in body size within a bioassay will also skew the results, indicating either greater or lesser sensitivity, depending on whether the significantly larger organisms were in the higher or the lower concentration. For instance, this discrepancy most likely skewed the data with the 1 September 1997 bioassay of *G. lacustris* so that the lower concentration, 0.4 ppm, appeared less toxic relative to the higher concentration of 1 ppm (Table 2).

Comparison of Marigold Root Extract with Toxicity of Other Pesticides on Macroinvertebrates. Two species, *R. morrisoni* and *D. grandis*, had a Hilsenhoff biotic index of 0 and 1, respectively, indicating they were the least tolerant of organic pollutants. Pollutants were defined here as those materials that create a high biochemical oxygen demand. In our studies, *R. morrisoni* and *B. tricaudatis* were the least tolerant, with the former having succumbed to mortality even in controls and the latter dying within 24 h of exposure to the inert ingredients at 0.01 ppm. The least tolerant of both the marigold root extract and the inert materials alone were these same two species, *R. morrisoni* and *B. tricaudatus*, along with H. cockerelli (which was not listed in the Hilsenhoff biotic index). The ranking of species from most to least tolerant in the Hilsenhoff biotic index was *B. tricaudatis* (4) (this species was not given in the Hilsenhoff index, but the other *Baetis* species had an index between 4 and 5) > G. lacustris (4) (this species was not given in the Hilsenhoff index, but the other *Gammarus* species had an index of (4) > *L. pluviale* (1) > *D. grandis* (1) > *H. cockerelli* (1) (this *Hydropsyche* species was not listed in the Hilsenhoff index). Those *Hydropsyche* species listed were classified from 0 to 6 by Hilsenhoff. We compared ours to *H. phalerata* which Hilsenhoff classified as (1) > *R. morrisoni* (0). The ranking of species, as indicated by LT_{50}s (Table 2) from most to least tolerant to both the marigold root extract and inert materials, was *G. lacustris* > *L. pluviale* > *H. cockerelli* > *D. grandis* > *B. tricaudatis* > *R. morrisoni.* The ranking of species from most to least tolerant to the inert materials alone was slightly different: *G. lacustris* > *H. cockerelli* > *L. pluviale* > *D. grandis* > *B. tricaudatis* > *R. morrisoni.* Another plant-derived compound of interest yielded similar results. The ranking for most to least tolerent for the neem formulation (Dunkel and Richards 1998) in the Hilsenhoff biotic index is: *Caecidotea* spp. > *S. parallela* > *D. grandis* and *B. occidentalis* > *D. doddsi* > *B. americanus.* The ranking of species from the most to least tolerant to both the neem formulation and inert materials from the LT_{50}s was: *D. grandis* > *Caecidotea* spp. > *S. parallela* > *B. americanus* > *D. doddsi* > *B. occidentalis.* The LC_{50} for *D. grandis* obtained in late winter and exposed to a 3% formulation of azadirachtin that included neem kernel extract (35%) and inert ingredients (65%) was 7.15 ppm (Dunkel and Richards 1998). The LC_{50} for *D. grandis* obtained from the same location in early spring and exposed to a formulation of *T. minuta* root extract plus the same inert as with the neem formulation was 6.91ppm (Table 1).

Our results with formulated marigold root extract indicated that this natural material was four to five orders of magnitude less toxic for aquatic macroinvertebrates than some synthetic insecticides related to natural compounds. For example, fenvalerate, a synthetic pyrethroid, had LC_{50}s ranging from 0.00003 (at 96 h) to 0.00093 ppm at 24 h (Anderson 1982, Smith and Stratton 1986) and flucythrinate, another synthetic pyrethroid, had LC_{50}s of 0.00022 ppm at 96 h (Anderson and Shubat 1984).

Macroinvertebrate Toxicity of Inert Ingredients in Formulation. A marigold root extract formulation with inert concentration at 0.01 ppm would be ideal for protecting nontarget macroinvertebrates in aquatic environments. Further studies should be conducted testing the inert concentration at our given value (0.01 ppm) with other plant-based compounds to assess the economic and biological viability of using a potential pesticidal plant.

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References Cited

Anderson, R. L. 1982. Toxicity of fenvalerate and permethrin to several non-target aquatic invertebrates. Environ. Entomol. 11: 1251–1257.

Anderson, R. L., and P. Shubat. 1984. Toxicity of flucythrinate to *Gammarus lacustris* (Amphipoda), *Pteronarcys dorsota* (Plecoptera) and *Brachycentrus americanus* (Trichoptera): importance of exposure duration. Environ. Pollut. Ser. A Ecol. Biol. 35: 353–365.

Davide, G. R. 1979. Effects of nematocides and *Tagetes erecta* on the control of *Meloidogyne incognita* and on yield of tomato. Philipp. Phytopathol. 15: 141–144.

Dosdall, L. M., M. M. Galloway, J. T. Arnason, and P. Morand. 1991. Field evaluation of the phototoxin, alpha-terthienyl, for reducing larval populations of black flies (Diptera: Simuliidae) and its impact on drift of aquatic invertebrates. Can. Entomol. 123: 439–449.

Dunkel, F. V., and D. C. Richards. 1998. The effect of azadirachtin formulation on six nontarget aquatic macroinvertebrates. Environ Entomol. 27: 667–674.

Finney, D. J. 1952. Probit analysis. Cambridge University, New York.

Graham, K., E. A. Graham, and G.H.N. Towers. 1980. Cercaricidal activity of phenylheptatriyne and alpha-terthienyl, naturally occurring compounds in species of Asteraceae (Compositae). Can. J. Zool 58: 1955–1958.

Green, M. M., J. M. Singer, D. J. Sutherland, and C. R. Hibben. 1993. Larvicidal activity of *Tagetes minuta* (marigold) toward *Aedes aegypti.* J. Am. Mosq. Control Assoc. 7: 282–286.

Hilsenhoff, L. W. 1987. An improved biotic index of organic stream pollution. Great Lakes Entomol. 20(1): 31–39.

Ijani, M.S.A., and M. T. Mmbaga. 1988. Studies on the control of root-knot nematodes (*Meloidogyne* species) on tomato in Tanzania using marigold plants (*Tagetes* species), ethylene dibromide and aldicarb. Trop. Pest Manage. 34: 147–149.

Miller, M. P., and J. F. Ahrens. 1969. Influence of growing marigolds, weeds, two covercrops and fumigation on subsequent populations of parasitic nematodes and plant growth. Plant Dis. Rep. 53: 642–646.

Nowierski, R. M., Z. Zeng, S. Jaronski, F. Delgado, and W. Swearingen. 1996. Analysis and modelling of time-dose-mortality of *Melanoplus sanguinipes, Locusta migratoria migratorioides* and *Schistocera gregaria* (Orthoptera: Acrididae) from *Beauveria, Metarhizium* and *Paecilomyces* isolates from Madagascar. J. Invertebr. Pathol. 67: 236–252.

Perich, J. M., C. Wells, W. Bertsch, and K. E. Tredway. 1994. Toxicity of extracts from three *Tagetes* against adults and larvae of yellow fever mosquito and *Anopheles stephensi* (Diptera: Culicidae). J. Med. Entomol. 31: 833–837.

Philogene, B.J.R., J. T. Arnason, C. W. Berg, F. Duval, and P. Morand. 1985. Efficacy of the plant phototoxin alpha-terthienyl against *Aedes intrudens* and effects on non-target organisms. J. Chem. Ecol. 12: 893–898.

SAS Institute. 1988. SAS technical report P-179, additional SAS/STAT procedures, release 6.03. SAS Institute, Cary, NC.

Smith, T. M., and G. W. Stratton. 1986. Effects of synthetic pyrethroid insecticides on non-target organisms. Residue Rev. 97: 93–120.

Weaver, D. K., C. D. Wells, F. V. Dunkel, W. Bertsch, S. E. Sing, and S. Sriharan. 1994. Insecticidal activity of foliar and root extracts of *Tagetes minuta* (Asterales: Asteraceae) against adult Mexican bean weevils (Coleoptera: Bruchidae). J. Econ. Entomol. 87: 1718–1725.

Weaver, D. K., L. J. Zettler, C. D. Wells, J. E. Baker, W. Bertsch, and J. E. Throne. 1997. Toxicity of fractionated and degraded Mexican marigold floral extract to adult *Sitophilus zeamais* (Coleoptera:Curculionidae). J. Econ. Entomol. 90: 1678–1683.

Wells, C. D., A. Mongin, and W. Bertsch. 1993. A study of photosensitive insecticidal volatiles in marigold (*Tagetes minuta*). J. High Resolut. Chromatogr. 16(1): 53–55.

Welty, L., and L. Prestbye. 1993. Determination of feasibility of using rotational crops to reduce pest problems in peppermint. Report of 2-year project. Montana State University Northwestern Agricultural Experiment Station, Kalispell, MT.

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