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Manuka Oil, A Natural Herbicide with Preemergence Activity

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Natural herbicides approved in organic agriculture are primarily nonselective burn-down essential oils applied POST. Multiple applications are often required due to their low efficacy. To address this problem, the in vivo herbicidal activity of manuka oil, the essential oil distilled from manuka tree (*Leptospermum scoparium* J.R. and G. Forst), was tested on selected broadleaf and grass weeds. While manuka oil exhibited good POST activity when applied in combination with a commercial lemongrass oil–based herbicide, it ultimately demonstrated interesting PRE activity, providing control of large crabgrass seedlings at a rate of 3 L ha^{-1}. Manuka oil and its main active ingredient, leptospermone, were stable in soil for up to 7 d and had half-lives of 18 and 15 d, respectively. The systemic activity of manuka oil addresses many of the current limitations associated with natural herbicides. Additionally, its soil persistence opens up a multitude of new possibilities for the use of manuka oil as a tool for weed management and may be a potential bridge between traditional and organic agriculture.

Nomenclature: Leptospermone; 2,2,4,4-tetramethyl-6-(3-methyl-1-oxobutyl)-1,3,5-cyclohexanetrione, CAS 567-75-9; large crabgrass; *Digitaria sanguinalis* (L.) Scop. DIGSA.

Key words: Natural product, triketone, phytotoxins, herbicide, mode of action, *p*-hydroxyphenylpyruvate dioxygenase, essential oil, organic agriculture.

Weeds have a greater negative impact on crop yields than any other agricultural pests (Oerke 2006). As a result, conventional agricultural practices rely on highly effective synthetic herbicides for managing weeds, and these compounds account for more than half of the volume of all agricultural pesticides applied in the developed world. Public sentiment toward synthetic herbicides has grown increasingly negative, and new pesticide regulations (i.e., Food Quality Protection Act, 1996) have contributed to a reduction of the number of new products commercialized by the major agrochemical manufacturers (Gerwick 2010). This has had a particularly negative impact on minor crop producers (Gast 2008).

Demand for organic food, on the other hand, has grown tremendously throughout the developed world (Dimitri and Oberholtzer 2009). Organic farming does not permit the use of synthetic pesticides (EPA 2011). Natural alternatives that are currently approved for organic agriculture are mostly nonselective essential oils used as POST, burn-down products. Their low efficacy requires multiple applications of high amounts to achieve good weed control (Young 2004). The process is expensive both in terms of the cost of the material applied and the cost of the applications. There is, therefore, a need for new natural weed management tools.

Two of the major limitations of natural herbicides approved for organic use are their lack of systemic activity and their nonspecific mechanisms of action (Dayan and Duke 2010). In an effort to develop new natural herbicides with superior properties, we recently reported that several natural products including the natural β-triketones present in manuka oil have the same molecular target site as the commercial synthetic herbicides sulcotrione and mesotrione, namely the enzyme *p*-hydroxyphenylpyruvate dioxygenase (HPPD) (Dayan et al. 2007; Meazza et al. 2002; Romagni et al. 2000).

HPPD is a key enzyme in the biosynthesis of tocochromanols (e.g., tocopherols and tocotrienols) and prenyl quinones (e.g., plastoquinone). Since plastoquinone is a cofactor necessary for phytoene desaturase function (Norris et al. 1995), HPPD inhibitors indirectly cause a deleterious reduction in the levels of carotenoids in plants. Subsequently, the reduced pool of carotenoids is not sufficient to quench the excess electrons generated during photosynthesis, causing a rapid degradation of chlorophylls (photodynamic bleaching) (Pallett et al. 1998).

While a structure–activity relationship study demonstrated that the length of the aliphatic side chain of the natural triketones of manuka oil modulated the activity of the compounds (Dayan et al. 2009b), the efficacy of β-triketone–rich manuka oil as a herbicide has not been proven, much less optimized. Hence, it was hypothesized that this essential oil may have in vivo herbicidal activity. Therefore, manuka oil was tested on selected broadleaf and grass weeds, and its effects on chlorophyll, carotenoids, and biomass were measured to determine its PRE and POST activity. The soil stability and bioavailability of manuka oil and its primary component leptospermone (Figure 1) were studied to understand the basis for the PRE activity of this oil.

Materials and Methods

Chemical Supplies. Manuka oil was obtained from Clean & Green Trading Co.[1] The nonionic sticker-spreader Nu-Film P (96% poly-1-*p*-menthene) approved for organic farming was purchased from Miller Chemical & Fertilizer Corporation[2] and the lemongrass oil natural herbicide GreenMatch EX™ (lemongrass oil [50%], CAS 8007-02-1, and a mixture of water, corn oil, glycerol esters, potassium oleate and lecithin) was obtained from Marrone Bio Innovations.[3] All other chemical supplies were purchased from Sigma-Aldrich.[4]

Synthesis of Leptospermone. Dry phloroglucinol (10–12 mM) was added to a solution of phosphorus oxychloride (15 ml) and anhydrous aluminum chloride (4 g) and stirred

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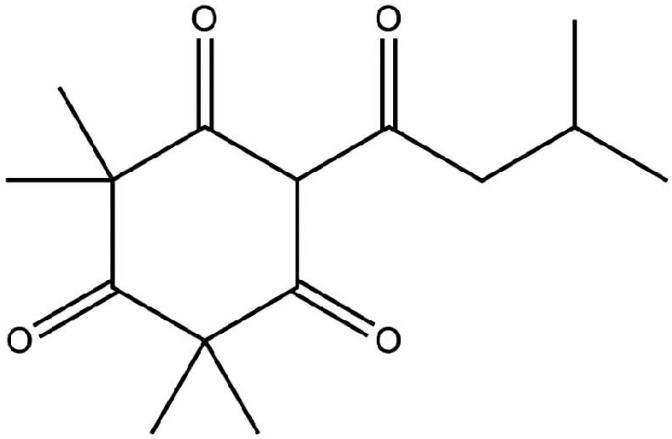

Figure 1. Structure of leptospermone, the primary active ingredient of manuka oil.

under nitrogen gas (N_2). A solution of 2-methyl butanoic acid (10 mM) was added, and the reaction mixture was stirred at 0 C for 8 h under N_2 and in a cold room (ca. 6 C) for a further 40 h. The mixture was poured onto crushed ice (ca. 100 g) and extracted with diethyl ether (2 × 100 ml). The ether extract was washed with saturated sodium bicarbonate (500 ml) and dried over anhydrous magnesium sulfate ($MgSO_4$). The diethyl ether was removed using a rotary evaporator to yield an oily residue which was applied to a silica gel column (40 g) that had been preequilibrated with cyclohexane-ethyl acetate (5 : 1). Fractions containing mostly di- and triacyl products eluted first, followed by the target monoacylated product in yields between 40 and 54%. The monoacylated phloroglucinol was preleptospermone [3-methyl-1-(2,4,6-trihydroxyphenyl)-1-butanone, CAS# 26103-97-9].

Sodium methoxide was prepared by dissolving 0.3 g sodium in 5 ml methanol. Methyl iodide (3 ml) was added to the sodium methoxide solution followed by the addition of mono-acyl phloroglucinol (1.5–2.5 mM) under N_2. The reaction mixture was heated under reflux for 3 h. The solvent was evaporated under vacuum and the extract acidified with 1 M HCl (50 ml) and extracted with diethyl ether (3 × 50 ml). The ether phase was extracted with 5% sodium carbonate (Na_2CO_3) (200 ml). The Na_2CO_3 extract was acidified with concentrated HCl, extracted with diethyl ether (2 × 200 ml) and dried over anhydrous $MgSO_4$ to yield the triketone (yields between 70 and 85%). Final purification was achieved by high-vacuum bulb-to-bulb distillation (1 mm Hg, 150 C). The product matched the reported physical data values of leptospermone.

Properties of the Soil Used in the Studies. All POST experiments were carried out in Metro-Mix 350 potting soil.[5] Studies on the PRE activity and soil stability of manuka oil were carried out in a Commerce silty clay loam soil collected in a field that has never been treated with herbicides near the USDA Jamie Whitten Research Center in Stoneville, Mississippi (coordinates: 33°25′22.68″N, 90°53′52.9″W). The soil characteristics were as follows: fine-silty, mixed, superactive, nonacid, thermic Fluvaquentic Endoaquept, 38.5% sand, 47.75% silt, 13.75% clay, organic matter 1.08%, pH 6.4, 35 μg g^{-1} Mg, 8.2 μg g^{-1} K, and 92.8 μg g^{-1} Ca. The soils were air-dried, sieved (0.5 mm), and stored at room temperature.

Seeds, Growth of Plants, and Spraying Apparatus. Barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] seeds were obtained from Dr. David Gealy (USDA Dale Bumpers National Rice Research Center, Stuttgardt, Arkansas); redroot pigweed (*Amaranthus retroflexus* L.) seeds were purchased from Herbiseed[6]; all other seeds, velvetleaf (*Abutilon theophrasti* Medik.), field bindweed (*Convolvulus arvensis* L.), large crabgrass, and hemp sesbania [*Sesbania exaltata* (Raf.) Rydb. ex A.W. Hill], were purchased from Azlin Seed Service.[7]

For all experiments, plots were set up using 10-cm-diameter plastic pots in trays without drainage holes and watered as needed from the bottom throughout the course of all the experiments. Plants were grown in the greenhouse at ambient temperatures with supplemental lighting to provide 16 h daylight per day.

All herbicide applications were performed with a Generation III Spray Booth[8] equipped with a model TeeJet EZ 8002 nozzle[9] with conical pattern and 80° spray angle. The height from nozzle to soil level was 40 cm for all the experiments. The spray head was set to move over the plants at 1.5 km h^{-1} and the apparatus was calibrated to deliver the equivalent of 360 L ha^{-1}.

POST Activity of Manuka Oil on Selected Weeds. Seeds of velvetleaf, redroot pigweed, field bindweed, large crabgrass, barnyardgrass, and hemp sesbania were planted to determine germination rates and specific times required for each species to emerge. Once established, six pots of each weed species were initiated at staggered intervals to provide seedlings that would be at a similar developmental stage (two true leaves for monocots, four true leaves for dicots) at the same time. Plants were thinned to three seedlings per pot prior to treatment.

POST application consisted of 10% (v/v) GreenMatch EX or 10% (v/v) GreenMatch EX plus 1% (v/v) manuka oil applied using the spray chamber described above. All treatments, including the control, contained 0.5% Nu-Film P. Individual plant heights were measured 2 wk after treatment. The aboveground portions of the plants were harvested and their fresh weight was measured. Samples were dried in paper bags at 60 C for a minimum of 48 h and plant dry weights were recorded.

Effect of Staggered POST Treatment with Manuka Oil. To establish whether staggered spraying enhanced the efficacy of manuka oil, pots were seeded with large crabgrass as before and thinned to three plants per pot. Treatments were 10% GreenMatch EX, 1% manuka oil, 10% GreenMatch EX and 1% manuka oil applied together, 10% GreenMatch EX and 0.5% manuka oil applied together followed by a second application of 0.5% manuka oil 1 wk after the first application, and 10% GreenMatch EX followed by 1% manuka oil 1 wk after the first application. All treatments, including control, contained 0.5% Nu-Film P. Plant height, dry weight, and chlorophyll content were measured.

Preemergence Activity of Manuka Oil. Having observed that some of the late-germinating seeds in manuka oil–treated pots were emerging from the soil with bleaching symptoms, dose–response curves were determined for manuka oil applied PRE. Pots were filled with the agricultural soil and seeded as described above. Twenty-four hours later pots were sprayed with manuka oil at 0, 0.1, 0.25, 0.5, and 1%, suspended in

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water with 0.5% Nu-Film P. Three pots were treated at each concentration. Seedlings were not thinned for these experiments. Two weeks after treatment, individual measurements were taken of each plant's height with plants from each pot counted and bagged for dry weight determination.

Quantification of Chlorophylls, Carotenoids, and Biomass. Total carotenoids were extracted by homogenizing 5 mg of leaf tissue in 3 ml of 6% (w/v) potassium hydroxide in methanol using a Polytron PT-3100.[10] Following centrifugation at 3000 *g*, the extract was partitioned in 3 ml of diethyl ether : benzene (1 : 9) and 1.5 ml of saturated sodium chloride. Carotenoid concentrations were determined as micrograms of carotenoids per gram of fresh weight after measuring absorbance at 445 nm in the organic phase according to Sandmann and Böger (1983) using an extinction coefficient of E445 = 2,500 (% w/v).

Total chlorophylls were extracted from 10 mg of leaf tissue in 3 ml of dimethyl sulfoxide at 62 C for 2 h (Hiscox and Israelstam 1979). Absorbance of the extracts was read at 645 and 663 nm using a UV-3101 PC.[11] Chlorophyll concentrations were determined according to Arnon (1949) and converted to milligrams of chlorophyll per gram of fresh weight of sample. Biomass was measured as fresh weight at the time of harvest and as dry weight (after the samples were dried in an oven at 65 C for 48 h).

High Performance Liquid Chromatography (HPLC) Analysis of Leptospermone. The HPLC system used to measure leptospermone extractable from soil was composed of a Waters Corporation system[12] which included a Model 600E pump, a Model 717 autosampler, a Millenium 2010 controller, and a Model 996 photodiode detector equipped with a 3.9 mm by 30 cm Waters µbondapak C18 reversed phase column. The solvent system consisted of an isocratic baseline at 63 : 37 acetonitrile : H_2O from 0 to 12 min, followed by an 8-min wash with 100% acetonitrile and a 10-min re-equilibration to the initial conditions before the next injection. The flow rate was 1 ml min^{-1} and the injection volume was 50 µl. A calibration curve was established by injecting various concentrations of pure leptospermone.

Soil Stability of Leptospermone. The soil stability of leptospermone was studied in a multisolute system (manuka oil) and in a single-solute system (pure leptospermone) in the agricultural soil characterized above. The initial manuka oil amount in the multisolute system was 20 µg g^{-1} of soil, which is equivalent to 7 µg of leptospermone per gram of soil. Consequently, the amount of leptospermone used in the single-solute system was 7 µg g^{-1} of soil.

The experiment consisted of 5 g of soil in 20-ml glass vials with foam stoppers to facilitate air circulation. The soil moisture was maintained at 80% field capacity. The vials were incubated in complete darkness at 24 C and 95% relative humidity. The control consisted of double-distilled water with no leptospermone or manuka oil. Quintuplicate samples were extracted with 5 ml of distilled water at 0, 1, 3, 7, 21, and 28 d after incubation. The extracts were centrifuged for 20 min at 3,300 *g*, filtered through a 0.45-µm nylon membrane filter[13] and analyzed by HPLC as described above. There was no background amount of leptospermone in the control native soil.

Statistical Analysis. Data from dose–response and time-course experiments were analyzed using the dose–response curve module (Ritz and Streibig 2005) using R version 2.2.1 (R Development Core Team 2009). Analysis of the means was performed using the PROC GLM of the SAS statistical software program[14] and means were separated by Fisher's LSD Test at $\alpha = 0.05$.

Results and Discussion

Essential oils used in organic agriculture, such as lemongrass oil (e.g., GreenMatch EX), clove oil (e.g., Burnout™), pine oil (e.g., Organic Interceptor™), and citrus oil (e.g., Organic Weed & Grass Killer™), are strictly fast-acting POST contact formulations. They generally disrupt the cuticular layer of the foliage which results in the rapid dessication or burn-down of young tissues (Dayan et al. 2009a). However, lateral meristems tend to recover and additional applications of essential oils are necessary to control the regrowth (Young 2004). Commercial essential oils must be applied at high concentrations, often 10% or more per volume, that correspond to 50 to 500 L of active ingredient per hectare (Dayan and Duke 2010).

Manuka oil differs from other essential oils in that it contains large amounts of several natural β-triketones (Dayan et al. 2007; Douglas et al. 2004; Hellyer 1968). The oil and its primary β-triketone component leptospermone, are strong inhibitors of HPPD, with I_{50} values of 15 and 3 µg ml^{-1}, respectively (Dayan et al. 2007). Therefore, the ability of a

Table 1. Effect of manuka oil on the herbicidal activity of GreenMatch EX against a variety of monocotyledonous and dicotyledonous weeds. GreenMatch EX was applied at the recommended rate of 10% (v/v) and manuka oil as supplemented as 1% (v/v).

Species	Pigweed	Velvetleaf	Bindweed	Hemp sesbania	Large crabgrass	Barnyardgrass
	Plant height (cm)[a,b]					
Control	19.9 ± 2.2 a	32.5 ± 3.1 a	41.6 ± 5.5 a	30.6 ± 2.4 a	45.8 ± 7.2 a	60.0 ± 5.1 a
10% Green Match EX	16.5 ± 5.5 a	18.1 ± 5.0 a	10.4 ± 5.3 b	11.4 ± 2.9 b	30.5 ± 7.4 b	49.8 ± 5.9 b
10% GreenMatch EX + 1% manuka oil	9.9 ± 2.0 b	14.1 ± 6.3 b	6.27 ± 4.7 b	10.0 ± 3.3 b	24.6 ± 2.1 b	42.4 ± 6.1 c
	Plant dry weight (g)[a,b]					
Control	0.76 ± 0.22 a	1.75 ± 0.36 a	0.97 ± 0.17 a	1.73 ± 0.29 a	1.85 ± 0.62 a	1.90 ± 0.42 a
10% Green Match EX	0.57 ± 0.27 a	1.07 ± 0.45 b	0.14 ± 0.07 b	0.28 ± 0.18 b	1.25 ± 0.44 b	1.62 ± 0.20 a
10% GreenMatch EX + 1% manuka oil	0.26 ± 0.05 b	0.76 ± 0.55 b	0.07 ± 0.05 b	0.21 ± 0.13 b	0.50 ± 0.08 c	0.80 ± 0.38 b

[a] Means of six replications and error bars represent the standard deviation.
[b] Numbers in columns followed by the same letters are not statistically different according to Fisher's LSD Test at $\alpha = 0.05$.

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Table 2. Pigment content in large crabgrass new leaves 2 wk after POST application of GreenMatch EX alone or with manuka oil.[a]

Treatment	Chlorophyll	Carotenoids
	mg g^{-1} FW	μg g^{-1} FW
Control	1.52 ± 0.16 a	1,438.64 ± 88.97 a
GreenMatch EX (10%)	0.67 ± 0.16 b	1,330.71 ± 285.92 a
GreenMatch EX (10%) + manuka oil (1%)	0.03 ± 0.01 c	312.27 ± 92.61 b

[a] Values are the means of five replications followed by standard deviation. Numbers in columns followed by the same letters are not statistically different according to Fisher's LSD Test at $\alpha = 0.05$.

small amount (1% v/v) of manuka oil to augment the activity of the commercial organic herbicide GreenMatch EX (lemongrass oil) applied at its recommended rate (10% v/v) was tested on several monocotyledonous and dicotyledonous weeds. In all cases, manuka oil seemed to potentiate the POST weed control obtained with GreenMatch EX alone, but the dry weights were significantly smaller only for pigweed and the grass weeds large crabgrass and barnyardgrass (Table 1). The dicotyledonous weeds exhibited mostly the burn-down phenomenology commonly associated with lemongrass oil treatment (Dayan et al. 2009a; Dayan and Duke 2010); whereas, the monocotyledonous weeds also exhibited the bleaching effect of the triketones on the regrowth. The dry weight of large crabgrass was particularly affected by the combination of GreenMatch EX and manuka oil.

Therefore, large crabgrass was selected as a model monocotyledonous species to investigate the effect of POST application of manuka oil and GreenMatch EX on the chlorophyll and carotenoid contents of the regrowth. Plants recovering from the initial burn-down effect of GreenMatch EX had lower chlorophyll content than the controls, but their carotenoid content was not affected (Table 2). On the other hand, large crabgrass plants recovering from the combination of GreenMatch EX and manuka oil were bleached and contained less chlorophylls and carotenoids compared with GreenMatch EX alone. Though these seedlings survived in the greenhouse, their growth was stunted and most likely would be less competitive against other plants in a field situation.

Table 3. POST activity of GreenMatch EX and manuka oil applied alone or in combination on large crabgrass seedlings.[a]

Treatment[b]	Height[c]	Dry weight[c]	Chlorophyll[d,e]
	cm	mg	mg g^{-1} FW
1	26.97 ± 1.55 a	0.33 ± 0.05 a	1.38 ± 0.05 a
2	15.72 ± 4.00 bc	0.13 ± 0.05 b	1.03 ± 0.17 b
3	17.27 ± 2.75 b	0.15 ± 0.05 b	0.91 ± 0.11 bc
4	10.06 ± 1.94 d	0.02 ± 0.01 c	0.44 ± 0.10 de
5	10.39 ± 1.70 d	0.03 ± 0.01 c	0.29 ± 0.06 e
6	12.78 ± 5.06 cd	0.07 ± 0.04 c	0.66 ± 0.28 cd

[a] Numbers in columns followed by the same letters are not statistically different according to Fisher's LSD Test at α=0.05.

[b] 1 = control; 2 = 10% GreenMatch EX; 3 = 1% manuka oil; 4 = 10% GreenMatch EX + 1% manuka oil; 5 = 10% GreenMatch EX + 0.5% manuka oil followed by 0.5% manuka oil 1 wk after treatment; 6 = 10% GreenMatch EX followed by 1% manuka oil 1 wk after treatment.

[c] Means of six replications followed by standard deviation.

[d] Means of three replications followed by standard deviation.

[e] Abbreviation: FW, fresh weight.

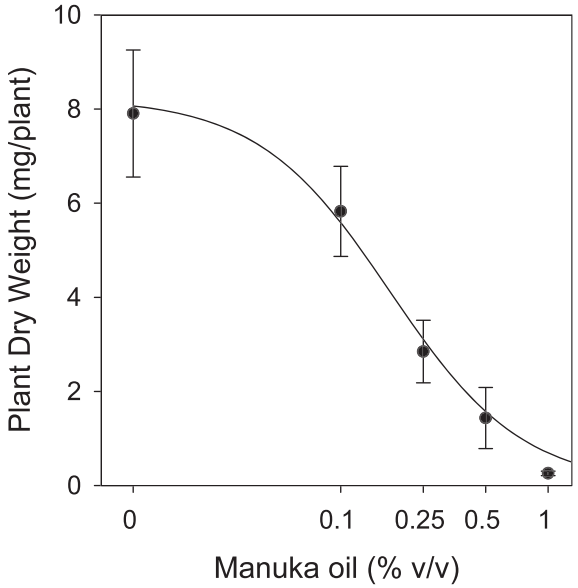

Figure 2. Dose–response curve of manuka oil applied PRE. Weights were taken 2 wk after treatment. Data represents means of three replications and the standard deviation. I_{50} and I_{90} values of the PRE activity of manuka oil on large crabgrass were 0.17 ± 0.03% and 0.87 ± 0.26% (v/v), respectively, as calculated from a three-parameter logistic regression obtained using the dose–response curve module in R version 2.2.1.

In light of the responses observed with individual applications of manuka oil and GreenMatch EX on large crabgrass, another experiment was designed to test the effect of the timing of manuka oil application on the potency of GreenMatch EX. Both GreenMatch EX or manuka oil applied alone caused approximately 40, 60, and 30% reductions in large crabgrass height, dry weight, and chlorophyll content, respectively (Table 3). However, the combination of 10% GreenMatch EX and 1% manuka oil applied together was much more potent, causing as much as a 94% reduction in large crabgrass dry weight. Similar results were obtained by applying 0.5% manuka oil with the initial GreenMatch EX treatment, followed by spraying another 0.5% manuka oil a week later. However, this approach requires two separate applications instead of a single application, with no improvement in weed control. Finally, spraying 1% manuka oil a week after the application of 10% GreenMatch EX did not improve the control of large crabgrass, relative to when applied together.

During this experiment, a few large crabgrass seeds germinated after the POST application of manuka oil, and these seedlings emerged from the soil completely bleached. This unexpected observation suggested that manuka oil may remain in soils for extended periods of time, enabling it to have PRE activity. Therefore, a dose response curve was

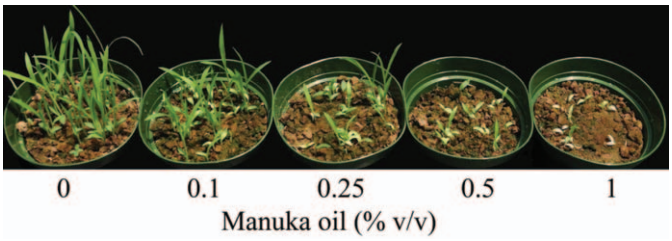

Figure 3. Dose–response curve of manuka oil on large crabgrass. Manuka oil was dissolved in water containing 0.5% Nu-Film P. Photo was taken 2 wk after treatment.

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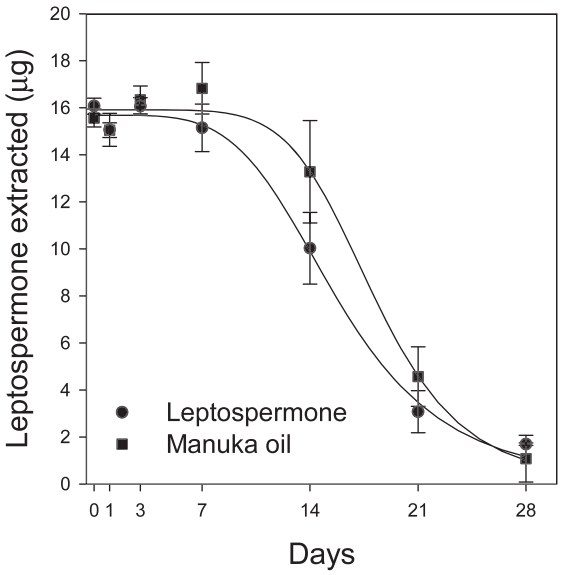

Figure 4. Soil stability of leptospermone applied alone (single-solute system) or as a component of manuka oil (multisolute system). Data represents means of five replications and the standard deviation. The time required for 50% degradation ($T_{1/2}$) of leptospermone was 15.8 ± 0.4 d in the single-solute system and 18.2 ± 0.4 d in the multisolute system as calculated from three-parameter regression curves using the dose–response curve module in R version 2.2.1. The $T_{1/2}$ values were statistically different from each other at P = 0.0001.

obtained with various concentrations of manuka oil applied PRE.

The PRE application of manuka oil resulted in a good control of large crabgrass (Figure 2). Though the experiment was not designed to measure this, seed germination was not affected, but bleaching symptoms increased with rates of application (Figure 3). Based on the dose–response curve calculated using a three-parameter logistic regression, the growth of large crabgrass was inhibited by 50 and 90% with 0.17 and 0.87% manuka oil, respectively. This corresponds to 0.6 and 3.1 L ha^{-1} manuka oil or 0.2 and 1.1 L ha^{-1} leptospermone, the primary active ingredient of the oil. Albeit obtained in a greenhouse experiment, this level of activity is superior to all other currently available essential oils commercialized for weed control. Most products require 50 to 500 L of active ingredient per hectare (Dayan and Duke 2010). Furthermore, most essential oils require multiple applications to control the regrowth of plants following the initial burn-down effect (Dayan et al. 2009a), but manuka oil appears to have a more systemic herbicidal mode of action, observed as bleaching of the foliage. Therefore, regrowth of the surviving plants may not be as much of an issue with this essential oil.

The strong PRE activity of leptospermone raised the question of the persistence of this compound in soil. This was tested by measuring the amount of leptospermone that could be extracted from a native soil from the Mississippi delta. Approximately 23% of the leptospermone applied to the soil was extractable in the aqueous phase during the first 7 d of incubation in the dark at room temperature. The levels decreased with little to none remaining after 28 d of incubation (Figure 4).

Tharayil et al. (2008) found that the biological activity of allelochemicals in soil was enhanced when they were applied as a mixture. Similarly, the half-life of the active ingredient is longer when applied as a component of manuka oil than alone. The $T_{1/2}$ of leptospermone was 15.1 ± 0.5 days when applied alone (single-solute system), and 18.2 ± 0.6 days when applied as manuka oil (multisolute system), according to a three-parameter regression analysis. The $T_{1/2}$ values were statistically different at P = 0.0001, suggesting that the application of leptospermone as a component of a mixture increases its persistence in soil and may contribute to its PRE activity.

In conclusion, manuka oil can be used to potentiate the herbicidal activity of other herbicidal essential oils. Further research will be conducted to determine if this effect is the result of additive or synergistic actions. However, the prospect of developing manuka oil, or its principle active ingredient leptospermone, as a novel natural herbicide with PRE activity is particularly attractive. The appeal of "greener" technologies has begun to impact conventional agriculture with renewed interest in natural product discoveries. Indeed, natural herbicides present themselves as a potential bridge between traditional and organic agriculture (Dayan and Duke, 2010; Dayan et al. 2009a), and major agrochemical companies are pursuing and developing new environmentally friendlier weed management tools. The serendipitous discovery of the PRE activity of manuka oil and the further characterization that leptospermone possesses some soil persistence presents a multitude of new possibilities for the use of manuka oil as a tool for weed management in both organic and conventional farming systems. Future research into the PRE activity of manuka oil on other monocot and broadleaf weeds is needed to further explore the bioactivity and selectivity of this natural herbicide.

Sources of Materials

[1] Manuka oil, Clean & Green Trading Co., Felton, CA 95018.
[2] Nu-Film P, Miller Chemical & Fertilizer Corporation, Hanover, PA 17331.
[3] GreenMatch EX™, Marrone Bio Innovations, Davis CA 95618.
[4] Chemical supplies, Sigma-Aldrich St. Louis, MO 63103.
[5] Potting soil, Sun Gro Horticulture, Bellevue, WA 98008.
[6] Redroot pigweed seed, Herbiseed, Twyford, England, United Kingdom RG10 0NJ.
[7] Velvetleaf, field bindweed, large crabgrass, and hemp sesbania seed, Azlin Seed Service, Leland, MS 38756.
[8] Generation III Spray Booth, track sprayer, DeVries Manufacturing, Hollandale, MN 56045.
[9] Teejet nozzle, Spraying Systems Co., Wheaton, IL 60189.
[10] Polytron PT-3100, Kinematica, Inc., Bohemia, New York, NY 11716.
[11] UV-3101 PC, Shimadzu Corporation, Kyoto, Japan.
[12] Waters Corporation system, Milford, MA 01757.
[13] Nylon membrane filter, Nalgene Company, Rochester, NY 14602.
[14] SAS statistical software program, Version 10, SAS Institute Inc., 100 SAS Campus Drive, Cary, NC 27513.

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