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# N, P and K Fertilizers Alter Plant Growth, Essential Oil Yield and Gender of Sweet Gale (*Myrica gale* L)

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**Abstract:** To investigate whether the fertilizers N, P or K individually affect plant growth, oil content and the gender of sweet gale, two trials, pot and field trials, were carried out at Orkney College UHI in Scotland. A pot trial was established with eight soils which were collected from different sweet gale trial sites in the north of Scotland. Although neither shoot yield nor oil concentration in shoots was affected by soil, there were significant differences in shoot yields as a result of fertilizer treatments (nitrogen (N), phosphorus (P), potassium (K) or none (control)). The best yield was obtained from the N treatment which was double to that of the control and P treatments. N, P or K fertilizers did not consistently affect shoot oil concentration in two seasons; however, oil yield was significantly affected, and N treatment produced two-three fold oil yield increases compared with the control or P treatment. In the N treatment, the increase in shoot yield was positively correlated with total nitrogen or nitrate nitrogen in the soil, suggesting the occurrence of a nitrogen priming effect. Data suggested that as shoot yield increased the oil concentration in shoots decreased. Neither soil nor N, P or K fertilizers had a significant effect on oil composition. Amongst fertilizer treatments, P resulted in the largest number of plants changing gender from female to male. A field N trial confirmed that nitrogen significantly enhanced the shoot yield of young plants.

**Keywords:** Essential Oil, gender, growth, *Myrica gale*, NPK.

## 1. INTRODUCTION

The genus *Myrica* includes about 60 species and has a worldwide distribution. The word *Myrica* is derived from Greek, *myron*, meaning perfume [1, 2]. Sweet gale (*Myrica gale* L.) is native to north-west Europe, North America and Asia [2]. It was reported that European *M. gale* populations are hexaploid while North American ones are dodecaploid [3]. Sweet gale is found throughout the UK except the Channel Isles and Shetland. Its use in brewing started about 2000 years ago in northern Holland, a practice which has been spread to many other places [1, 4]. It is still used for flavouring of spirit today. It has been used as an insect repellent (antifeedant) for centuries, particularly for midges [1, 2, 5-7]. The leaves of *M. gale* are dried to perfume linen. The catkins or cones are boiled in water to produce scum beeswax for making candles. The roots and barks are used to tan calfskins or dye wool if collected in autumn. Dried nuts are used as a spice. The leaves are also used as stomachic and cordial medicines in China and for the treatment of dermal disease and dysentery in France [1]. Recent research suggests that the volatile oil has unique anti-bacterial effects and is highly resistant to oxidation [7, 8], and has anticancer activity [9].

In Scotland, *M. gale* flowers from March to May on the wood of the previous year's growth, before the leaves are produced. The scented flowers are dioecious (individual flowers are either male or female, but only one sex is to be

found on any one plant so both male and female plants must be grown if seed is required). Yellow-green or short red catkins appear in leaf axils in early summer, pollination is by the wind, and the plants are not self-fertile [1]. The staminate flowers are developed much earlier than pistillate catkins. This plant is occasionally monoecious and can also change sex from year to year, possibly as a result of nutrition [10], although this has not been investigated.

*M. gale* is able to obtain all its Nitrogen by N<sub>2</sub>-fixation and grows vigorously in N-free nutrient solutions [11]. It also can take up P from wet, poor soil where N and P may be deficient by cluster roots which consist of dense clusters of determinate rootlets [1]. Root nodules can fix Nitrogen and this can have a significant impact on the communities on which it occurs [1] and can increase forest productivity on peat soils [12]. However, Camp [13] reported that although nutrient availability in good fen peat soils is sufficient for seedling establishment, plant growth is still enhanced by supplementary nutrients suggesting that the nutrient level in the wild fen peat is not optimal for *M. gale* growth. As a part of a project to investigate the potential for growing *M. gale* as an agricultural crop, the trials in this paper were established to investigate whether the fertilizers N, P or K individually affect plant growth, oil content and the gender of *M. gale*.

## 2. EXPERIMENTAL

There were two trials, a pot trial and a field trial which were carried out by the Agronomy Institute at Orkney College UHI in Kirkwall, Scotland (N58°58' W02°59'). The field trial was established to verify the pot trial results.

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## 2.1. Pot Trial

Eight different soils were collected from sweet gale planting sites in the north of Scotland (Table 1).

There were eight pots of each soil which were planted (one plant per pot) on 15 July 2008 (18 cm diameter pots, 4 litres) and re-potted in 25 cm diameter pots (8 litres) on 8 March 2010. Plants were three year old seedlings obtained from Alba Trees Ltd, UK. Plants were maintained in an open-ended polythene tunnel for five months and then moved outside in January 2009. Each plant pot was placed in its own saucer and from April to September plants were watered regularly to ensure that water was present in the saucer.

On 21 July 2008, plant height and stem diameter were measured to check the uniformity of plants before applying fertilizer treatments. The measurements showed that there were no significant differences in plant height (mean, 46.8 cm; cv, 7.5%), and stem diameter measured at 5 cm above the soil (mean 6.2 mm; cv, 14.6%).

## 2.2. Fertilizer Treatment

There were four fertilizer treatments applied to this trial, i.e. control (no fertilizer), N, P and K (Table 2). For each soil, there were two pots with each treatment. Therefore, there were sixteen pots for each fertilizer treatment and 64 pots in total. The amount of fertilizer applied per pot was based on a plant density at 40,000 plants per hectare. After fertilizer application, pots were completely randomized as 8 x 8 rows.

Fertilizer applications were made on 27 August 2008, 20 April 2009, 24 August 2009, 6 April 2010 and 7 June 2010.

## 2.3. Plant Cutting Back and Harvesting

Plants were cut back to 30 cm on 20 April 2009 before leaf emergence and harvested at 32 cm above the soil on 11 August 2009 and 12 August 2010. Therefore the harvesting did not include woody stems from the previous year's growth. At harvesting, the fresh weight of shoots per plant was recorded, and a mixed fresh shoot sample of two plants

**Table 1. Characteristics of soils from eight sweet gale trial sites in Scotland used in the pot trial.**

Characteristic*	Alvie N57°09' W03°53'	Black Isle N57°38' W04°04'	Blair Atholl N56°46' W03°56'	Craibstone N57°11' W02°13'	Elgin N57°35' W03°21'	Glen- borrodale N56°41' W05°45'	Kirkton N56°25' W04°39'	Kirkwall Orkney N58°58' W02°59'
pH	4.7	5.2	6.8	5.3	5.2	4.3	4.9	5.2
P (mg l <sup>-1</sup> )	8.40	31.70	13.50	62.90	25.30	11.30	45.20	37.40
K (mg l <sup>-1</sup> )	35.00	136.00	68.00	75.00	35.00	72.00	69.00	42.00
Mg (mg l <sup>-1</sup> )	29.00	100.00	21.00	117.00	34.00	59.00	55.00	93.00
Nitrate N (mg kg <sup>-1</sup> )	0.50	1.90	2.16	6.94	2.99	1.91	4.88	9.91
Ammonium N (mg kg <sup>-1</sup> )	1.42	2.68	2.59	1.61	1.21	3.75	1.87	1.21
Available N (kg ha <sup>-1</sup> )	7.65	18.25	18.95	34.20	16.80	22.60	27.05	44.45
Total N (%w w <sup>-1</sup> )	0.16	0.25	0.32	0.33	0.16	0.39	0.37	0.36
Sand %	53.00	53.00	51.00	55.00	56.00	80.00	68.00	47.00
Silt %	37.00	37.00	34.00	30.00	32.00	16.00	26.00	38.00
Clay %	10.00	10.00	15.00	15.00	12.00	4.00	6.00	15.00
Texture	Sandy Loam	Sandy Loam	Sandy Loam	Sandy Loam	Sandy Loam	Loamy Sand	Sandy Loam	Sandy Silt Loam

\*The soils were analysed by NMR Ltd.

**Table 2. Fertilizers and doses of each application.**

Fertilizer	NPK Composition	N,P or K (kg ha <sup>-1</sup> )	Fertilizer (kg ha <sup>-1</sup> )	Fertilizer (g pot <sup>-1</sup> )
Control	N/A	N/A	N/A	N/A
Sulphate of Ammonia	20-0-0	25	125.00	3.13
Superphosphate	0-17-0	25	147.00	3.63
Sulphate of Potash	0-0-48	25	52.10	1.30

from the same treatment was collected for oil concentration and composition analysis.

## 2.4. Field Nitrogen Trial

To determine whether field-grown plants responded to N, a field nitrogen trial was established. Planting was carried out on 9 March 2009 at Muddisdale (Agronomy Institute Field), Kirkwall in Orkney and used plants of two heights, i.e. 7-9 cm (from BritishFlora, UK) and 20-40 cm (from Quercus Garden Plants Ltd, UK) which are referred to as P1 and P2, respectively. Plant spacing was 50 cm by 50 cm. Plot size was 2 m by 2.5 m with 20 plants per plot. All plants were cut back at 20cm above the ground in March 2010 to standardise their canopy height. Kerb flo (3.75 Lha<sup>-1</sup>) and Roundup 360 (1.9 Lha<sup>-1</sup>) were applied on 8 February 2010 for weed control.

There were three nitrogen treatments: i.e. N0, N30 and N60 (0, 30 and 60 kg N/ha, respectively) and the application was split so that half was applied on 7 April and half on 14 June 2010. Sulphate of ammonia (NPK 20:0:0) was used for this trial. A split plot field design was used with five replicates; the main plot was nitrogen treatment and subplot was plant materials. Plants were harvested on 12 August 2010. At harvesting, shoot yield per plot was measured and a 100 g per plot fresh shoot sample of the P2 plant material was collected for oil concentration and composition analysis.

## 2.5. Oil Extraction and Analysis

Samples for oil analysis were put in plastic bags, stored in a cool box (ca 10 °C) then delivered to Inverness College UHI for analysis where all samples were immediately logged, given a sample number and then frozen at ca -20 °C prior to analysis. Samples were prepared for distillation by roughly crushing the leaves whilst in the bag to break them up. This was then emptied into a clean tray. Large stalks and unbroken leaves were cut up into smaller pieces. The sample was then transferred to a round bottomed distillation flask and the weight recorded to two decimal places and 450 ml of distilled water was added. Where samples were 50 g or less, 350 ml was added.

The flask was placed onto a heating mantle and connected to the distillation apparatus for one hr distillation. The heater was then switched off and the sample allowed to settle for a few minutes. The water level was slowly lowered by opening the tap on the collection arm until the oil collected could be measured against the graduation marks. The water was then run off slowly and the oil collected in a dark glass vial, labeled and measured.

The quantity of collected oil was converted to oil concentration (ml100g<sup>-1</sup>). Oil yield plant<sup>-1</sup> was calculated for each treatment as the product of shoot yield plant<sup>-1</sup> and oil concentration.

Oil samples were stored at ca 4 °C prior to analysis (normally within two days). 10 ul of oil was transferred by micropipette to another dark glass vial and diluted with 490 ul of ethyl acetate. The sample was then analysed by GC/MS (Hewlett Packard 5972 Mass Spectrometer with HP 5890 Series IIe Gas Chromatograph). Extracts were run on a 30 m x 0.25 mm ZB1 0.25 µm film column, mass spectra were

obtained in EI mode (70 eV) and scanning was from 35 - 500 amu. The injector and transfer line were kept at 250 °C and 280 °C respectively. The oven temperature was programmed from 60 °C to 246 °C at 3°C per minute, held initially at 60 °C for two minutes, then ramped to 246 °C where it was held for 10 minutes. Helium carrier gas was used at 1.02 ml min<sup>-1</sup>, injection volume was 1.0 µl.

Five standard solutions (0.1mg ml<sup>-1</sup> - 0.8mg ml<sup>-1</sup> - pinene, phellandrene, para cymene, 1,8-cineole, limonene) were used to generate standard lines and to check the linear range. For identification of peaks other than the five standard compounds, Adams (2001) was adopted and his spectra and retention times were used as references.

## 2.6. Statistical Analysis

An analysis of variance (ANOVA) table was calculated for shoot yield and oil content. Differences between treatments were assessed using the F-test, and the Least Significant Difference (LSD) was calculated at the 0.05 probability level.

## 3. RESULTS

### 3.1. Pot Studies

In the pot trial, neither shoot yield nor oil yield were significantly affected by different soils in 2009 or 2010 (Table 3). Averaged over soils, there was however a significant ( $P < 0.01$ ) negative correlation between shoot yield and oil concentration which reduced the oil yield of plants with high shoot yields (Fig. 1).

Shoot yield plant<sup>-1</sup> was significantly affected by fertilizer in both 2009 and 2010. The highest yield was obtained from the N treatment and it was about twice that of the control or P treatment. Although the shoot yields of the control and P treatment were not significantly different, the P treatment had slightly lower shoot yields in both years.

Oil content was significantly affected by the fertilizer treatment in 2009 but not in 2010. However, oil yield plant<sup>-1</sup> was significantly affected by fertilizer in both years (Table 4), and the N treatment produced a two to three fold increase in oil yield plant<sup>-1</sup> compared with the control or P treatment. As a result of having the lowest shoot yield and oil content, the P treatment produced the lowest oil yield, particularly in 2009 when this was significantly different to any other treatment.

In both years, linear regression analysis showed that the shoot yield in the nitrogen treatment was always significantly correlated ( $P < 0.001$ ) with soil available nitrogen or soil nitrate nitrogen content and, the regression lines accounted for 66% (Fig. 2) and 65% (Fig. 3), respectively, of the variations in shoot yield. There were no other significant correlations between yield and soil characteristics.

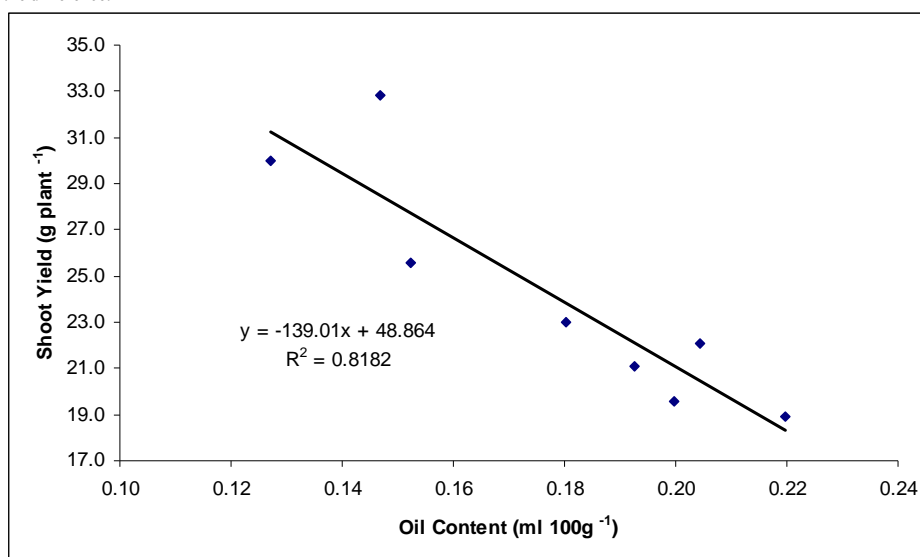
Although there was no significant interaction between fertilizer and soil on shoot yield plant<sup>-1</sup> ( $P = 0.990$ ) or oil yield plant<sup>-1</sup> ( $P = 0.994$ ) in 2009, interactions were significant in 2010 for both shoot yield (Fig. 4) and oil yield (Fig. 5).

For most soils, the highest yields were with the N treatment but for Alvie soil the control treatment did slightly

**Table 3.** Comparison of shoot and oil yields per plant in eight soils.

Yield	Year	Alvie	Black Isle	Blair Atholl	Craibstone	Elgin	Glenborrodale	Kirkton	Orkney	SED	Probability
Shoot Yield (g plant <sup>-1</sup> )	2009	22.1	18.9	32.8	25.6	30.0	19.6	23.0	21.1	8.820	0.722
	2010	21.0	17.0	27.0	26.5	30.5	17.9	25.8	31.1	11.420	0.110
Oil Yield (ml plant <sup>-1</sup> )	2009	0.045	0.041	0.048	0.035	0.038	0.042	0.044	0.038	0.0160	0.994
	2010	0.040	0.040	0.062	0.059	0.065	0.039	0.053	0.069	0.0120	0.354

SED: Standard error of the difference.

**Fig. (1).** Correlation between oil concentration and shoot yield (2009).**Table 4.** Comparison of shoot and oil yields under different fertilizer treatments.

Treatment	Fresh Shoot Yield (g plant <sup>-1</sup> )		Oil Content (ml 100g <sup>-1</sup> )		Oil Yield (ml plant <sup>-1</sup> )	
	2009	2010	2009	2010	2009	2010
Control	21.80	20.50	0.182	0.200	0.039	0.038
N	35.20	40.56	0.178	0.250	0.060	0.101
P	19.70	17.75	0.146	0.200	0.027	0.035
K	19.90	19.56	0.207	0.200	0.040	0.039
SED	4.28	3.96	0.017	0.047	0.001	0.008
Probability	0.003	< 0.001	0.007	0.676	0.013	< 0.001

SED: Standard error of the difference.

better than the N treatment. The highest yields were obtained from Orkney soil with N treatment which produced a shoot yield plant<sup>-1</sup> and oil yield plant<sup>-1</sup> of 77 g and 0.192 ml respectively, whereas, the lowest shoot yields (7 g plant<sup>-1</sup>) were obtained from Kirkton and Orkney soils with the K treatment, and Black Isle soil with the control or P treatment. The lowest oil yields were also obtained from the same soil and fertilizer treatments which produced 0.013-0.014 ml plant<sup>-1</sup>. Although in most soils, the K treatment reduced both shoot yield and oil yield, in Elgin and Craibstone soils this treatment enhanced yields com-

pared with the control, and the shoot yield was even slightly higher than that of the N treatment.

### 3.2. Field studies

The field N trial confirmed the pot trial result and demonstrated that N increased shoot yield, particularly of the younger P1 material where the N60 treatment produced about a three fold increase compared with the control treatment. Although the effect on the older material was not significant, the trend was the same as for the younger material (Table 5). The N30 treatment slightly increased the oil

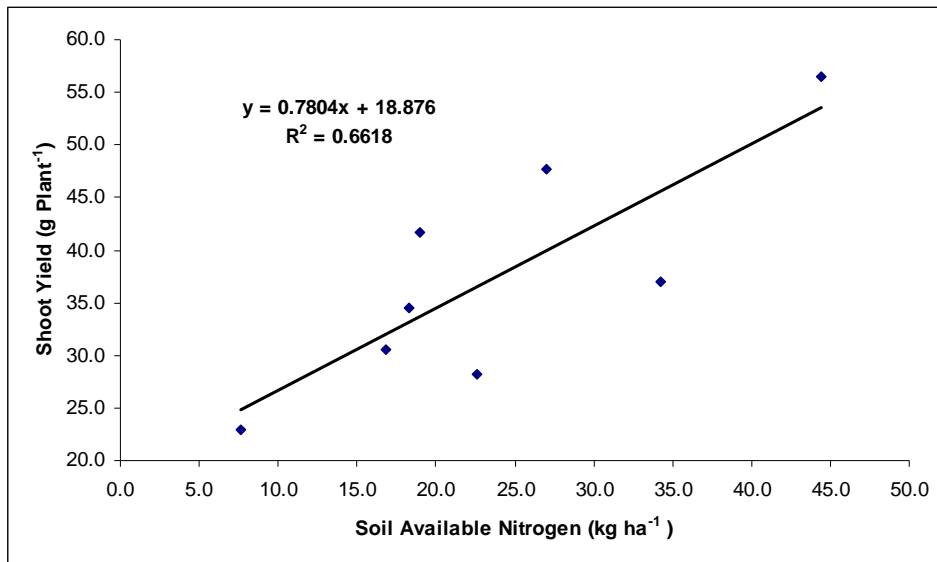


Fig. (2). Correlation between soil available nitrogen and the shoot yield of two year average under nitrogen treatment.

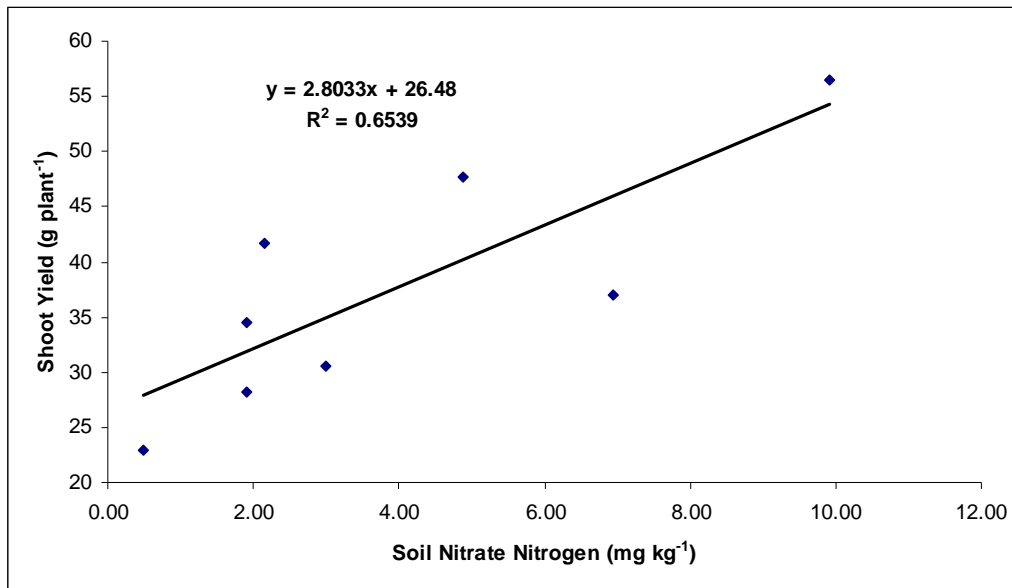


Fig. (3). Correlation between soil nitrate nitrogen and the shoot yield of two year average under nitrogen treatment.

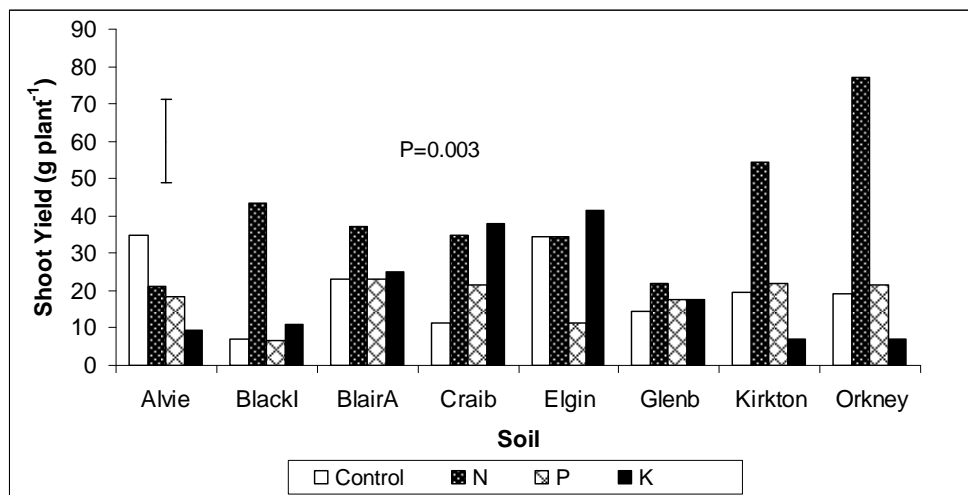


Fig. (4). Effect of soil and fertilizer treatments on shoot yield plant<sup>-1</sup> in 2010.

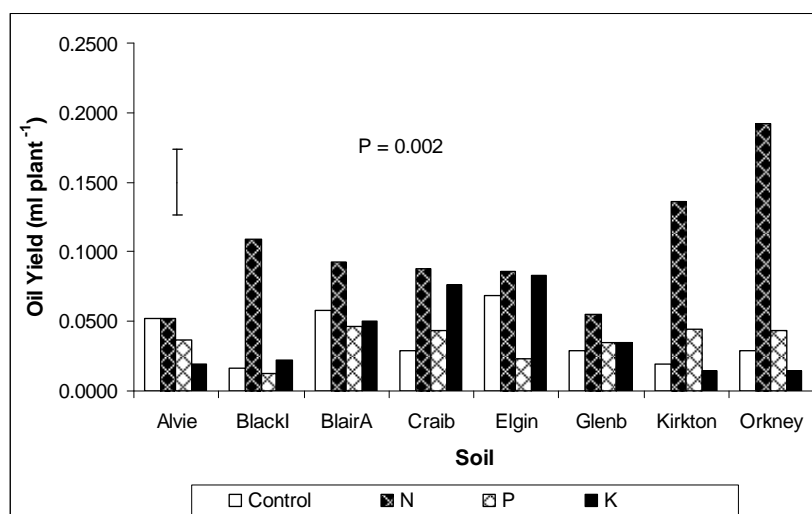


Fig. (5). Effect of soil and fertilizer treatments on oil yield plant<sup>-1</sup> in 2010.

Table 5. Shoot yield and oil concentration in the field under different N levels.

Treatment	Shoot Yield of P1 (g plant <sup>-1</sup> )	Shoot Yield of P2 (g plant <sup>-1</sup> )	Oil content of P2 (ml 100g <sup>-1</sup> )
Control	3.48	14.20	0.183
N30	5.71	15.50	0.233
N60	9.90	16.90	0.167
SED	2.216	3.570	0.024
Probability	0.039	0.770	0.068

SED: Standard error of the difference.

content of the P2 material although this was not significantly different to that of the control. When nitrogen was increased to 60 kg ha<sup>-1</sup> the shoot yield of the older plants (P2) slightly increased but the oil content decreased.

Eleven major compounds were identified and analysed in both the pot and field trials. None of them was significantly affected by soil or fertilizer treatment in the pot trial in either 2009 or 2010 (Table 6). In the field trial, although only alpha pinene was significantly increased by nitrogen there were non-significant, but quite large, increases in beta pinene, myrcene and phellandrene (Table 7). In contrast, germacrene and nerolidol were decreased, but not significantly.

In 2009, all the fertilizer treatments in the pot trial had a lower proportion of male:female plants (Table 8). By 2011, the control, K and N treatments showed a slight increase in the proportion of male:female plants which resulted in a more balanced sex ratio. This was very much more marked in the P treatment, however, where four plants changed from female to male (Table 8). These were from Craibstone, Kirkton, Black Isle and Blair Atholl soils which, apart from the latter, already had relatively higher phosphorus content (Table 1).

#### 4. DISCUSSION

This research suggested that Bog Myrtle can grow in agricultural soils with a range of characteristics, i.e. pH (4.3 -

6.8) in sandy loam and sandy silt loam soils (Table 1). Although the average shoot yields and oil yields of plants in the different soils were not significantly different, the response to the fertilizer treatments was different. Plants in Orkney soil were very responsive to N treatment whereas those in Alvie soil were not and most of those in the other soils were intermediate. It was also noticed that the shoot yield in the N treatment, but not in the control, was significantly correlated with soil available nitrogen or soil nitrate nitrogen. This may have resulted from a "nitrogen priming effect", interaction between fertilizer nitrogen and soil nitrogen [14], but needs further investigation.

Data from 2009 suggested that oil content decreased as shoot yield increased. This result agreed with previous field trial data suggesting a negative correlation between oil concentration and shoot yield per plant [15] due to dilution. Although oil content was not consistently affected by fertilizer treatment which was significant in 2009 but not in 2010, oil yield plant<sup>-1</sup> was significantly affected by fertilizer treatments in both years, and N treatment in the pot trial produced two to three times the oil yield plant<sup>-1</sup> of the control or P treatment. This result suggested that although sweet gale is able to obtain its nitrogen through N<sub>2</sub>-fixation and grows vigorously in N-free nutrient solutions [11], supplementary nitrogen is still very important to enhance the yield of biomass as suggested by Camp [13]. The nitrogen field trial

**Table 6. Retention times (RT) and relative content (%) of compounds identified in sweet gale shoot extracts from the pot trial in 2010.**

Compound	RT	Control	N	P	K	SED	Probability
Alpha pinene	6.070	19.05	19.10	17.61	18.19	3.051	0.942
Beta Pinene	7.260	2.79	2.68	2.51	2.72	0.761	0.976
Myrcene	7.739	4.58	4.99	3.29	2.48	2.888	0.813
Phellandrene	8.150	6.53	6.88	6.92	5.56	2.574	0.958
Para cymene	8.700	3.74	3.55	3.67	3.45	1.144	0.990
1,8 cineole	8.950	17.11	16.08	16.50	19.20	2.777	0.766
Limonene	9.040	10.81	10.41	9.88	9.37	1.704	0.939
Elemene G	25.790	1.42	2.33	1.50	2.97	1.360	0.606
Germacrene	30.380	6.94	10.66	9.02	16.1	6.708	0.531
Nerolidol	30.690	3.40	4.55	4.33	5.92	1.835	0.509
Germacrone	34.880	13.74	7.68	10.21	3.42	8.180	0.541

SED: Standard error of the difference.

**Table 7. Retention times (RT) and relative content (%) of compounds identified in sweet gale shoot extracts from the N field trial in 2010.**

Compound	RT	Control	N30	N60	SED	Probability
Alpha pinene	6.070	4.70	15.70	18.00	3.000	0.040
Beta Pinene	7.260	0.99	2.40	2.68	0.541	0.098
Myrcene	7.739	1.07	2.85	2.19	0.556	0.105
Phellandrene	8.150	3.38	6.44	6.87	1.409	0.157
Para cymene	8.700	1.64	2.53	3.00	0.685	0.278
1,8 cineole	8.950	9.50	12.00	17.50	3.370	0.198
Limonene	9.040	5.05	8.16	9.13	1.563	0.154
Elemene G	25.790	4.07	1.90	1.38	1.325	0.246
Germacrene	30.380	23.40	11.00	8.60	7.510	0.254
Nerolidol	30.690	12.70	9.00	6.20	3.650	0.334
Germacrone	34.880	15.90	15.60	10.30	7.570	0.733

SED: Standard error of the difference.

confirmed the pot trial result and also indicated that supplementary nitrogen was more effective on younger plants and may help early stage plant establishment. Results from water culture studies have shown that the form of N supply also affected the growth of sweet gale, and  $\text{NH}_4^+$  significantly enhanced the growth rate and shoot/root ratio, whereas, nitrate considerably reduced plant growth [16]. This needs further investigation in the field to confirm or otherwise. In contrast to the positive effect of N, the pot trial showed that the P treatment had the lowest shoot and oil content which resulted in the lowest oil yield.

In this research, the major oil compounds were alpha pinene, phellandrene, 1,8 cineole, limonene, germacrene and germacrone which is very different to the findings of Svoboda *et al.* [2], who reported the variation in chemical composition of the volatile oil of *M. gale* from various geographical sources, (i.e. Scotland, Finland, Eastern USA, Canada Ontario, Netherlands and Spain). They suggested that the major compounds in Scottish oil were alpha pinene, 1,8 cineole, beta elemone and germacrene, there was no trace of phellandrene and germacrone identified and the level of limonene was very low (1.5% of the relative content).



**Table 8.** Number and percentage of plants of different gender in each fertilizer treatment from 2009 to 2011.

Treatment	Gender	2009	2010	2011
CK	Male	6 (37.5%)	7 (43.8%)	7 (43.8%)
	Female	8 (50.0%)	8 (50.0%)	7 (43.8%)
	Male:Female	0.75	0.88	1.00
	Bisexual	1	1	2 (12.4%)
	Not-known	1	0	0
K	Male	6 (37.5%)	6 (37.5%)	6 (37.5%)
	Female	9 (56.3%)	8 (50.0%)	7 (43.8%)
	Male:Female	0.67	0.75	0.86
	Bisexual	0	1	3 (18.7%)
	Not-known	1	1	0
N	Male	6 (37.5%)	7 (43.8%)	7 (43.8%)
	Female	8 (50.0%)	8 (50.0%)	8 (50.0%)
	Male:Female	0.75	0.88	0.88
	Bisexual	0	1	1 (6.2%)
	Not-known	2	0	0
P	Male	5 (31.3%)	9 (56.3%)	10 (62.5%)
	Female	10 (62.5%)	6 (37.5%)	6 (37.5%)
	Male:Female	0.5	1.50	1.70
	Bisexual	1	1	0
	Not-known	0	0	0

Svoboda *et al.* [2], suggested that the oil composition differed markedly from one country to another. Differences between our results and those of Svoboda *et al.* [2] suggest that further research would be useful to document the oil composition in different locations in Scotland as the previous authors' sample was collected from Inchmarnock (55°47'N; 50°11'W), the latitude of which is about 350 km south of that of Orkney where the present research was conducted. Compared with the distillation methods used by Svoboda *et al.* [2], those in current research were different. In terms of the length of distillation, the previous research used 2 hr distillation while 1 hr distillation was used for the current research. This would make a significant difference as Sylvestre *et al.* [9], suggested that the length of distillation significantly affected the oil composition. Furthermore, the previous research used dried leaves for oil extraction and the current research used fresh leaves which could also make another significant difference.

Davey and Gibson [10] suggested that *M. gale* plant could change sex from year to year and this might be caused by nutrition, however, no further investigation of this has been done since then. Although this research observed that there were more *M. gale* plants altered from female to male in the phosphorus treatment, no definite conclusion could be drawn. This however may draw attention to scientists for in

depth investigation with field trials and in the wild to compare the content of nutrition in the soils and the sex ratios of populations, and then to understand the mechanism of the alteration.

The results of this research suggest that nitrogen fertilizer could be used to increase the shoot and oil yields of sweet gale grown on agricultural land, although the effect of this on the chemical composition of the oil needs further investigation.

#### CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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