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# The results of an experimental indoor hydroponic *Cannabis* growing study, using the 'Screen of Green' (ScrOG) method—Yield, tetrahydrocannabinol (THC) and DNA analysis

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ABSTRACT

The results of an indoor hydroponic *Cannabis* growth study are presented. It is intended that this work will be of assistance to those with an interest in determining an estimation of yield and value of *Cannabis* crops. Three cycles of six plants were grown over a period of 1 year in order to ascertain the potential yield of female flowering head material from such an operation. The cultivation methods used were selected to replicate typical indoor hydroponic *Cannabis* growing operations, such as are commonly encountered by the New Zealand Police. The plants were also tested to ascertain the percentage of the psychoactive chemical Δ-9 tetrahydrocannabinol (THC) present in the flowering head material, and were genetically profiled by STR analysis. Phenotypic observations are related to the data collected. The inexperience of the growers was evidenced by different problems encountered in each of the three cycles, each of which would be expected to negatively impact the yield and THC data obtained. These data are therefore considered to be conservative. The most successful cycle yielded an average of 881 g (31.1 oz) of dry, groomed female flowering head per plant, and over the whole study the 18 plants yielded a total of 12,360 g (436.0 oz), or an average of 687 g (24.2 oz) of dry head per plant. THC data shows significant intra-plant variation and also demonstrates inter-varietal variation. THC values for individual plants ranged from 4.3 to 25.2%. The findings of this study and a separate ESR research project illustrate that the potency of *Cannabis* grown in New Zealand has dramatically increased in recent years. DNA analysis distinguished distinct groups in general agreement with the phenotypic variation observed. One plant however, exhibiting a unique triallelic pattern at two of the five loci tested, while remaining phenotypically indistinguishable from three other plants within the same grow.



## 1. Introduction

### 1.1. The New Zealand environment

New Zealand is the most geographically isolated country in the world. It lies in the South Pacific Ocean, approximately 2250 km to the east of Australia and just to the west of the international dateline. While New Zealand is comprised of a group of narrow islands spanning nearly 13° of latitude, from the subtropical to the subantarctic, its main land areas experience a predominantly temperate climate, strongly influenced by the surrounding oceans. The population of just over 4.3 million people is highly urbanised, with over 86% living within the urban areas, which comprise just 2.7% of the total land area [1,2].

The remaining large areas of land, occupied by indigenous forests and reserves or under forestry or pastoral cultivation, provide ample opportunities for the covert cultivation of *Cannabis* crops outdoors, and to date most of the *Cannabis* seized in New Zealand has been grown outdoors. In the warmer, northern areas of New Zealand staggered planting allows the harvest of two crops per year, with a first phase of harvesting in early December producing a pre-Christmas crop which achieves very good prices, and a second phase of harvesting in February to March [3,4]. In most of New Zealand however, only one outdoor crop is possible annually, with the typical cultivation period being between September and March.

All drug-*Cannabis* in New Zealand has originally been illegally imported in the form of seed, with much of this either purchased

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over the Internet or from places such as the Netherlands where it can be legally purchased. The varieties claimed to be grown are therefore many of those familiar in other areas of the world—e.g.: 'Northern Lights', 'Hindu Kush', and 'Blueberry'. The risk of detection at New Zealand's border however, provides a strong incentive to propagate from selected seed-raised plants by the taking of cuttings, a process which is known as 'cloning'. The vast majority of indoor-grown *Cannabis* is grown from cloned plants. In recent years in New Zealand there appears to have been a shift with outdoor-grown *Cannabis* from seedlings, which were traditionally all that was found, to cloned plants; however there is some regional variation.

Indoor cultivation offers numerous advantages to the grower, including the ability to control environmental conditions and therefore grow continuously throughout the year. Over the last 10 years there has been a marked increase in indoor *Cannabis* growing operations discovered by the New Zealand Police (NZP). In 2006 7% of *Cannabis* plants seized by the NZP utilised hydroponics; which increased to 15% in 2007 [5].

The types of set-up vary widely in sophistication, from a single plant which may be grown in soil within a wardrobe area, to highly organised hydroponic operations being grown in commercial premises, such as the 2005 seizure from an Auckland warehouse occupied by the fashion label company, 'Insidious Fix', and the 2007 seizure in the Waitemata District involving 1750 *Cannabis* plants [6,7].

In a guideline judgement made by New Zealand's Court of Appeal, in the case R v Terewi [8], the court delineated three categories of offending in relation to *Cannabis* cultivation, each of which carries a suggested sentencing range. While prior to the Terewi case, sentences for cultivation tended to be based on the number of plants grown, this new judgement recognised the relatively new development of indoor, and especially hydroponic, cultivation, from which considerably enhanced yields are possible within the period of a year. The three 'Terewi categories' are:

Category 1: Cases where small quantities of *Cannabis* plants are grown, strictly for personal use. These are almost always assigned a fine or non-custodial sentence.

Category 2: Cases where there is small-scale cultivation of *Cannabis* for a commercial purpose. In these cases the sentence is usually between 2 and 4 years imprisonment.

Category 3: Cases where there is large-scale commercial cultivation of *Cannabis*, exhibiting both sophistication and organisation. The starting point for sentencing within this most serious category is usually 4 years or more.

In addition to the sentence imposed upon the offender, there is the provision in law to confiscate assets obtained through or used to facilitate the offending. New Zealand's Proceeds of Crime legislation (currently the *Proceeds of Crime Act 1991*, soon to be replaced by the *Criminal Proceeds (Recovery) Act 2009*, which comes into force on 1 December 2009) allows for the confiscation of the proceeds of serious criminal offending, where 'serious' offending (to be replaced with the phrase 'significant criminal offending') is defined as those offences which are punishable by 5 years or more in prison. To date this has been applied primarily to drug-related offending. The challenge for the prosecution is to provide proof of the value of the cultivation operations discovered, in order to enable the courts to calculate the potential income derived from these operations. While there have been a number of successful seizures [9], police have been hampered by a lack of empirical data on *Cannabis* plant yields with which to either inform the court or refute the defence's own claims regarding the potential value of the *Cannabis* plants or plant material seized, or to quantify the unlawfully derived income of the offender.

In 2004, a covert operation by Police of the Wellington Organised Crime Unit was terminated with the arrest of the operators of a hydroponic supplies shop and a number of their customers, who were found to have well-organised indoor *Cannabis* crops. This successful operation, referred to as 'Operation QUEEN', enabled the authors to examine a number of grow set-ups, many of which were using the same system, known as the 'Screen of Green' (ScrOG) method [10,11].

This paper presents the results of a research project, 'Operation REAP', which was jointly led by ESR and the NZP. In this study, three cycles of six *Cannabis* plants were grown hydroponically, under licence [12]. The purpose of the study was to ascertain the potential yield of female flowering head material, per year, from plants grown indoors using the ScrOG method. The operation REAP research project sought to replicate a typical indoor hydroponic *Cannabis* grow, such as is commonly encountered by the NZP. The yields obtained should not however, be applied directly to crops grown using methods which are substantially different from that used in this study.

The authors had no personal experience in the growing of *Cannabis* before this project. A convicted *Cannabis* grower assisted with the initial set-up and acted as an advisor throughout the study, on a confidential basis.

### 1.2. Taxonomic considerations

Until recently, *Cannabis* L. was considered to be one of only two genera in the family Cannabaceae; the other genus being *Humulus* (hops). A review of this and several related families within the Order Rosales has resulted in a previously separate family, the Celtidaceae, being absorbed into the Cannabaceae, thereby adding the genera *Aphananthe*, *Celtis*, *Chaetacme*, *Gironniera*, *Lozanella*, *Parasponia*, *Pteroceltis* and *Trema* [13].

Applying classical taxonomic methods to the genus *Cannabis* is highly problematic due to the long period of human cultivation, which has resulted in a very large number of phenotypically distinct taxa. Furthermore, the illegal nature of cultivation in most countries has led to a proliferation of informal names which may not be uniquely or universally applied.

The International Plant Names Index lists 13 validly published binomials for *Cannabis*: *Cannabis americana* Pharm. ex Wehmer; *Cannabis chinensis* Delile; *Cannabis erratica* Siev.; *Cannabis foetens* Gilib.; *Cannabis generalis* E.H.L. Krause; *Cannabis gigantea* Crevost; *Cannabis indica* Lam.; *Cannabis* × *intersita* Soják; *Cannabis kafiristanica* (Vav.) Chrtek; *Cannabis lupulus* (L.) Scop.; *Cannabis macrosperma* Stokes; *Cannabis ruderalis* Janisch., and *Cannabis sativa* L.

In recent years however, workers have examined the genus using molecular and chemical methods, and the prevailing taxonomic treatment is to regard *Cannabis* as a monospecific genus, containing only the highly variable species *Cannabis sativa* L. The species is widely regarded as having two subspecies, *C. sativa* subsp. *sativa* and *C. sativa* subsp. *indica*. The debate on the classification of *Cannabis* is well covered by Shao and Clarke [14] and by Gigliano [15], each following the arrangement proposed by Small and Cronquist [16], in their 1976 paper. An opposing opinion is given by Hillig and Mahlberg [17], who suggest the relative quantities of cannabinoid compounds present would support two species of *Cannabis*, *C. sativa* and *C. indica*, but this suggestion has not been generally adopted.

The taxonomy of *Cannabis* has important legal significance, as in many countries, including New Zealand, it is illegal to grow or possess the plant *C. sativa* [18,19]. However, since 2006, it has been legal in New Zealand, under licence from the Ministry of Health, to grow officially sanctioned varieties of fibre and seed-oil *Cannabis*

(industrial hemp) [20]. The hemp is legally required to contain less than 0.35% tetrahydrocannabinol (THC).

No published work exists on the varieties of psychoactive *Cannabis* available in New Zealand. This paper does not pretend to do more than provide an initiation into features which may be used to delineate the varieties present, by noting some phenotypic, physiological, chemical and molecular differences in the plants grown.

In addition to providing yield data, the plants grown were tested for the percentages of THC present in the mature floral heads, and DNA analysis was undertaken. These results are also presented here, along with a discussion of the findings.

## 2. Materials and methods

Detailed information regarding cultivation methods and materials, including the set-up, equipment required, nutrient and light regimes, and water and electrical usage are provided in the first paper in this series [21]. In order to address police operational concerns, this information is restricted to the law-enforcement community.

### 2.1. Growing conditions

Each crop of six plants was grown in a room with the dimensions of 4.32 m by 3.48 m and a height of 3.17 m. This area is comparable to the size of an average New Zealand bedroom, which is a space commonly used to grow *Cannabis* indoors.

The growing medium was inorganic, with commercially prepared nutrients and additives supplied in the water by an irrigation programme.

The plants for the first crop ('Grow 1') were purchased from an illegal grower. The second crop ('Grow 2') used six clones produced by taking cuttings of one of the plants from Grow 1. 'Grow 3' was of six plants taken from a clandestine growing operation discovered by the police.

The first two of the three crops used the ScrOG method, while the third grow was undertaken without the mesh screen, and heavy branches were supported simply by strings.

All equipment, nutrients and methods used were comparable to those commonly found at indoor growing operations at the time of the inception of the study.

### 2.2. Harvest and drying

The plants were harvested by cutting the branches from the main stem and hanging them to dry on strings within a secure laboratory. The branches were dried to the stage where stems of a pencil thickness snapped audibly when broken. At this point the plants had lost approximately 70–75% of their fresh weight. Plants can be dried to the point where they have lost 80–90% of their fresh weight, but this results in a loss of quality and is avoided by growers [22].

When dry, the plant material was groomed to remove any extraneous leaf and stem, with just closely cropped pieces of female flowering head remaining.

For Grows 2 and 3, the fresh and dried weights of the branches harvested from each plant were recorded as well as the groomed female flowering head material produced by each of the plants.

All plant material was weighed in grams, on a calibrated Mettler PE12 balance. Metric weights were converted to ounces and pounds using the website http://www.metric-conversions.org. The reason for this conversion is that the illicit drug market in New Zealand still predominantly operates using imperial measurements.

### 2.3. Δ-9 tetrahydrocannabinol (THC) analysis

Six randomly selected samples of head material from each plant in each grow were tested for THC concentration. The method of testing was an adaptation of the method developed in-house and reported by Poulsen and Sutherland [23]. This method enabled good separation of principal cannabinoids.

#### 2.3.1. Extraction of Cannabis plant

For each sample, approximately 2 g of dry female flowering head material was accurately weighed into a capped glass tube, noting the exact weight. Ethanol (10 ml) was added, ensuring that the plant material was totally immersed. The sample was then sonicated for 30 min.

#### 2.3.2. THC analysis

*Cannabis* extract solution (50 μl) was added to 450 μl ethanol and 500 μl internal standard solution directly into a gas chromatograph mass spectrometer (GCMS) vial. The internal standard was 4-androstene-3, 17-dione, at a concentration of approximately 0.2 mg/ml. Two THC standard stock solutions were prepared of Cerilliant T-005 (1 mg/ml), diluted to 0.1 and 0.2 mg/ml in ethanol. If the THC concentration of the plant samples tested fell outside the range of the two THC standard solutions, further dilution and injections were carried out.

#### 2.3.3. Instrumentation

Analysis was on a gas chromatograph: Agilent 6890 series with mass spectrometric detection using an Agilent HP-5ms, 30 m capillary column, with an inlet temperature of 280 °C and a detector temperature of 325 °C. The initial oven temperature was 80 °C with the following conditions: hold 1 min, ramp 50 °C/min to 300 °C, hold 9.6 min.

### 2.4. STR DNA analysis

Dried samples of leaf material from each of the Grow 1 and Grow 3 plants were genetically profiled using five trinucleotide short tandem repeat (STR) loci to determine whether the observed differences in phenotype, sexual expression and THC values were reflected in their genetic makeup. This work was done 'blind', in that the DNA analysts were not given information about any of the physical and chemical observations.

Duplicate samples of 100 mg of dried leaf material were taken from each of the 12 plants (six plants per grow). DNA was extracted from the samples using the DNeasy® Plant Mini Kit from Qiagen.

Known *Cannabis* leaf material and tomato (*Solanum lycopersicum*) leaf material positive controls and a negative (reagent only) control were also included, to monitor the DNA analysis process. The amount of DNA present in each extract was quantified using 1.5% agarose gel electrophoresis with ethidium bromide staining, using the Invitrogen High DNA Mass Ladder quantification standard.

The samples were then analysed using the five trinucleotide STR loci (Table 1) as previously published by Gilmore et al. [24].

Amplification products underwent capillary electrophoresis using an ABI PRISM® 3130 Genetic Analyser (Applied Biosystems Ltd.) and the raw data was analysed using GeneScan® v3.1 (AB). Fst Principal Coordinate Analysis (PCA) was carried out using GenAlEx v6 [25].

## 3. Results and discussion

### 3.1. Phenotypic observations

The first six plants obtained were said by the grower to be clones of the variety 'Red Devil'. In order to distinguish them for the purposes of data collection, they were numbered 'plant 1' to 'plant 6'.

Within a few weeks, it was apparent that there were at least two varieties present; Plants 1, 3, 4 and 6 were compact, bushy plants

**Table 1**
Description of loci.

| Locus | Repeat motif | Primer sequences | Expected amplicon size range |
|---|---|---|---|
| ANUC301 | $(TTA)_{15}$ | Fwd 5′-ATATGGTTGAAATCCATTGC-3′<br>Rvs 5′-TAACAAAGTTTCGTGAGGGT-3′ | 209–261 bp |
| ANUC302 | $(CAA)_7$–$(CAA)_4$ | Fwd 5′-AACATAAACACCAACAATGC-3′<br>Rvs 5′-ATGGTTGATGTTTTGATGGT-3′ | 140–173 bp |
| ANUC303 | $(GTG)_7$ | Fwd 5′-TAATCAACAATGACAATGGC-3′<br>Rvs 5′-GATTAAGGTCCTCGACGATA-3′ | 141–156 bp |
| ANUC304 | $(TCT)_8TCA(TCT)_7$ | Fwd 5′-TCTTCACTCACCTCCTCTCT-3′<br>Rvs 5′-TCTTTAAGCGGGACTCGT-3′ | 167–230 bp |
| ANUC306 | $(GAT)_3$–$(GAT)_6$ | Fwd 5′-ACTATTACTAAGCCTCCTCATCA-3′<br>Rvs 5′-GTGGTAGTCTCATTGTTGGTG-3′ | 92–95 bp |

**Fig. 1.** "*indica*-like" plant, Grow 1.

with large broad leaves—clearly of the habit described for *indica* varieties (Fig. 1), while Plants 2 and 5 had narrow leaflets and longer internodes resulting in taller, more openly branched plants, of the growth type referred to as indicative of *sativa* varieties (Fig. 2). Of particular interest was that each of the four *indica* phenotypes, while initially developing female floral heads, began to also produce some male flowers in week 15 of growth (2 weeks before harvest date). *Cannabis* is usually a strictly dioecious plant, with male and female flowers being produced on separate plants; monoecy and hermaphroditism can occur however, and are known to be induced by certain environmental factors [26–28] and also by the application of exogenous hormones, such as auxin and ethylene [29].

With the development of male flowers, and the consequent release of pollen, some of the adjacent flowers began to develop fruit, with the production of seeds (Fig. 3). As the plants were mostly mature, and seed production would greatly diminish the value of the product, it was decided to harvest the crop (Figs. 4 and 5). Although grown under exactly the same conditions, the *sativa*-like plants did not develop any male flowers.

Cuttings had been taken of both phenotypes; the sativa-like from 'plant 2' and the *indica*-like from 'plant 4' (Fig. 6). Due to the propensity of the *indica*-like phenotype to produce hermaphroditic flowers, the cuttings from 'plant 2' were used for the second grow.

The phenotype of the plants obtained from the police for the third grow was similar to the *indica*-like plants in Grow 1.

**Fig. 3.** Flowering head of "*indica*-like" plant, with male flowers, female flowers, and development of immature fruit, Grow 1.

**Fig. 4.** Mature plants, day 113 since planting clones, Grow 1.

**Fig. 2.** "*sativa*-like" plant, Grow 1.

**Fig. 5.** Harvested head from "*indica*-like" plant, Grow 1.

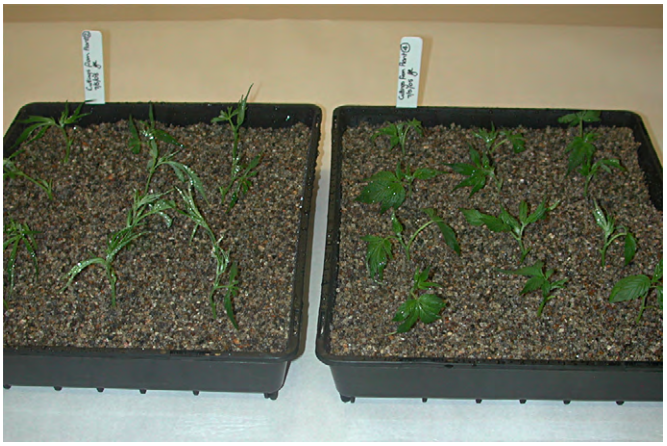

**Fig. 6.** Cuttings from "*sativa*-like" plant (left), and "*indica*-like" plant (right), Grow 1.

**Fig. 8.** Grow 3: spider mite web on flowering head.

### 3.2. Problems affecting yield and quality

Each of the three crops grown experienced a separate problem which impacted on the potential yield and quality of the *Cannabis* to some degree. For the second and third grows, the problems were serious, and reflected the inexperience of the researchers in growing *Cannabis*.

As discussed above, the first grow was harvested when male flowers developed on four of the plants, resulting in fertilisation of female flowers and the consequent onset of seed production. As this did not occur until the plants were mature, and the few immature seeds were still soft, any loss in quality was considered to be very minimal.

During the second grow, from cuttings taken from plant 2 of the first grow, a change in one of the additives used is suspected to have contributed to the nutrient burn of this crop (Fig. 7). By the time the problem was noticed, the leaves and bract material had irreversibly browned and begun to curl, causing floral development to be restricted. Flushing through with water resulted only in vegetative new growth; therefore the crop was harvested in the 17th week of the grow. This was approximately at the expected harvest date, but the floral heads had not developed to full maturity due to the stress the plants experienced in the previous 3 weeks.

The third grow was infected by the two-spotted red spider mite, which is a common pest of *Cannabis* as well as many other plants (Fig. 8) [30]. Examination of the root areas of these plants confirmed that they had been grown from cuttings, and were therefore clones [21]. The mites were not on the original clones, which had been obtained from a growing operation discovered by police in a south Wellington suburb. Unfortunately some infected plants, also sourced from a crime scene, were introduced into the grow room for temporary storage in the third week of the grow. These plants were removed and destroyed about 2 weeks later when mites were seen on them. It was not noticed that the crop had been contaminated until adult mites were seen in the 10th week of the round. By that time the plants were large, having completed the vegetative phase of the cycle and were well into the flowering phase.

Two-spotted mites lay eggs underneath the leaves and upon hatching, migrate up to the distal extremity of the branches and spin webs. Due to the density of the plant growth by the time the infestation was recognised, spraying with mite-killing agent was ineffective, as it could not reach all the under-leaf surfaces. Spider mites are leaf-sucking pests, which pierce the cells and draw out the fluid within, causing the leaves to mottle, dry out and die. As the developing heads are on the terminal ends of branches, they were being shrouded with the mite's webs. Although the leaves and also the bracts within the floral heads were being damaged, the flowers themselves appeared to only be affected by the loss of the nutrient flow which would normally be supplied by the leaves and bracts. The decision was made to harvest at 14 weeks of growth, approximately 2 weeks prior to the expected harvest date.

**Fig. 7.** Grow 2: nutrient burn.

### 3.3. Yields

The groomed female flowering head from the first grow was packaged in one-ounce quantities in zip-lock plastic bags, a common quantity and packaging found to be used by indoor growers. This 'wholesale' quantity is usually broken down further by the retailers, producing for example, $50 bags, which contain approximately 3 g, and 'tinnies'—or metal foil-wrapped quantities of approximately 0.5–1 g and sold for $20–25 each, although $20 is the most common value [31]. The number of full ounce bags obtained from each of the plants in this grow ranged from 25 to 47, and there was a total of more than 186 oz produced. Although the yields of the second and third crops were reduced, the average yield over the three crops was 687 g (24.2 oz, or just over 1.5 lb) of dried female flowering head per plant. Yields obtained from each of the three crops grown are presented in Tables 2–4, and in Fig. 9.

**Table 2**
Grow 1: yield of *Cannabis* female flowering head.

| Plant no. | Dry groomed head | | |
|---|---|---|---|
| | g | oz | lb |
| 1 | 868 | 30.62 | |
| 2 | 1340 | 47.27 | |
| 3 | 672 | 23.70 | |
| 4 | 781 | 27.55 | |
| 5 | 896 | 31.61 | |
| 6 | 728 | 25.68 | |
| Total | 5285 | 186.43 | 11.65 |

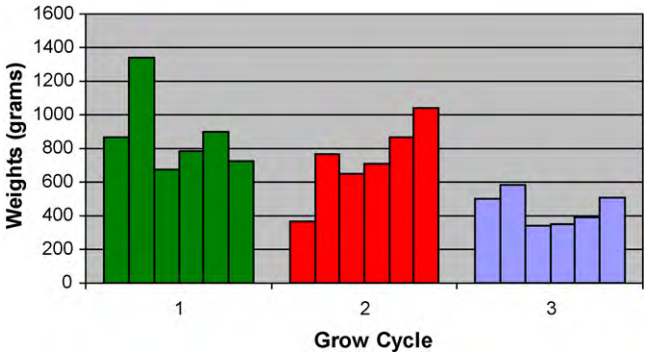


**Fig. 9.** Yield of each of six plants in each of three grows.

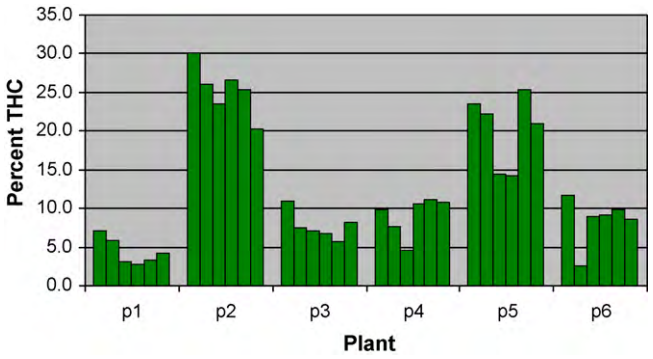


**Fig. 10.** THC results for six randomly selected samples from each of six plants in Grow 1.

The approximate value of these grows, in New Zealand dollars using 2009 prices, is given in Table 5.

### 3.4. Δ-9 tetrahydrocannabinol (THC) values

It was found that there was considerable variation present both within and between plants (Figs. 10–15). This finding is in agreement with that noted by Fiddian and Quinn [32] and by Hassan and Somerville (pers. comm., June 2009). This wide intra-plant and inter-plant variation is unsurprising, given the narrow window of time in which a flower is in peak condition. All the

**Table 3**
Grow 2: yield of *Cannabis* female flowering head, also fresh and dry weights of cut branches.

| Plant no. | Fresh weight of cut branches (g) | Dried weight of cut branches (g) | Dry branches as % wet weight (g) | Dry groomed head | | |
|---|---|---|---|---|---|---|
| | | | | g | oz | lb |
| 1 | 5673 | 1375 | 24 | 370 | 13.05 | |
| 2 | 7532 | 2594 | 34 | 769 | 27.13 | |
| 3 | 4661 | 1120 | 24 | 653 | 23.03 | |
| 4 | 5467 | 1334 | 24 | 708 | 24.97 | |
| 5 | 4684 | 1160 | 25 | 863 | 30.44 | |
| 6 | 4926 | 1237 | 25 | 1041 | 36.72 | |
| Total | 32,983 | 8820 | ($\bar{x} = 27$) | 4404 | 155.34 | 9.71 |

**Table 4**
Grow 3: yield of *Cannabis* female flowering head, also fresh and dry weights of cut branches.

| Plant no. | Fresh weight of cut branches (g) | Dried weight of cut branches (g) | Dry branches as % wet weight (g) | Dry groomed head | | |
|---|---|---|---|---|---|---|
| | | | | g | oz | lb |
| 1 | 3635 | 949 | 26 | 500 | 17.64 | |
| 2 | 4731 | 1228 | 36 | 581 | 20.49 | |
| 3 | 3709 | 1025 | 28 | 340 | 11.99 | |
| 4 | 4503 | 1185 | 26 | 350 | 12.35 | |
| 5 | 4962 | 1259 | 25 | 389 | 13.72 | |
| 6 | 6752 | 1718 | 25 | 511 | 18.03 | |
| Total | 28,292 | 7364 | ($\bar{x} = 26$) | 2671 | 94.22 | 5.89 |

**Table 5**
Value of *Cannabis* female flowering head from study, related to the quantity sold.

| | Sold as | | | |
|---|---|---|---|---|
| | Pounds $3500–5000 | Ounces $250–500 | $50 bags (3 g) | Bullets/foils/tinnies (0.5–1 g) $20 |
| Grow 1 | $40,775–58,250 | $46,608–93,215 | $88,083 | $105,700–211,400 |
| Grow 2 | $33,985–48,550 | $38,835–77,670 | $73,400 | $88,080–176,160 |
| Grow 3 | $20,615–29,450 | $23,555–47,110 | $44,517 | $53,420–106,840 |
| Total yearly value | $95,375–136,250 | $108,998–217,995 | $206,000 | $247,200–494,400 |

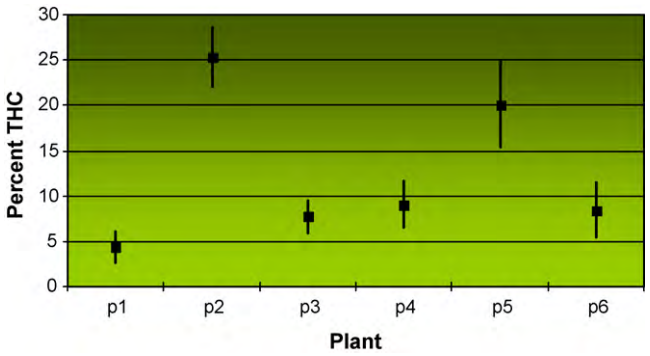


**Fig. 11.** The mean THC, with a band of ± one standard deviation, of six THC measurements, for each plant of Grow 1.

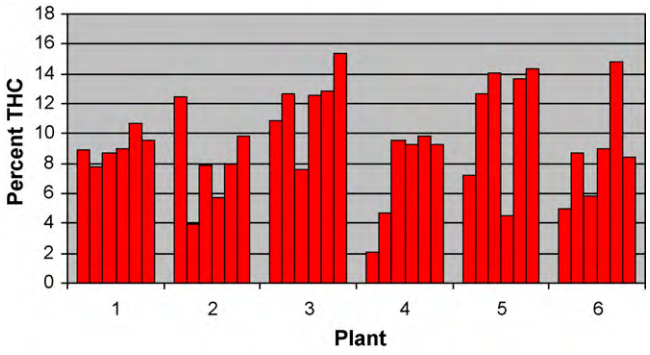


**Fig. 12.** THC results for six randomly selected samples from each of six plants in Grow 2.


**Fig. 13.** Mean THC results for each of the six plants in Grow 2.

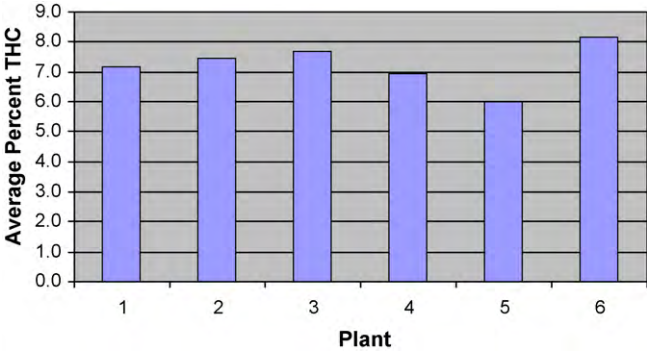


**Fig. 14.** THC results for six randomly selected samples from each of six plants in Grow 3.

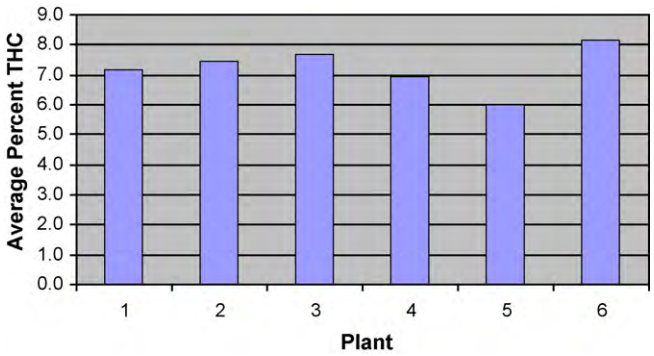


**Fig. 15.** Mean THC results for each of the six plants in Grow 3.

flowers present in a particular head are unlikely to be at exactly the same stage of ripeness; even far less likely that all the flowers on a plant will be synchronised in their degree of maturation. As plants are almost invariably harvested at one time, the resulting product is a mixture of flowers at slightly differing stages of THC production. This result should be considered by all researchers undertaking THC analyses, as it is clear that if multiple analyses are not performed on each sample, there is a risk of obtaining a result which is quite unrepresentative of the true average potency of the sample.

Due to the serious problems encountered in the second and third grows, only the THC values obtained from the analysis of the plants from the first grow are likely to reflect 'true' levels. This opinion is reinforced by the results from the second grow, which although grown under conditions very similar to those used for Grow 1, produced average THC levels of less than one third of those found in the Grow 1 plant from which they had been cloned.

The Grow 1 THC results are of great interest for two reasons: first, the maximum potency measured, 30% THC, was more than three times greater than the maximum potency ever previously reported in New Zealand, which was for a single sample of outdoor-grown *Cannabis* head which measured 9.7% THC [23].

Secondly, the Grow 1 THC results display a clear distinction between the potency of the two phenotypically distinct groups, which have been referred to above as "*sativa*-like" and "*indica*-like". The two "*sativa*-like" plants (plants 2 and 5) had average potencies of 20% and 25.2%, while the four "*indica*-like" plants had average potencies ranging from 4.3 to 9.2% THC (Figs. 10 and 11). As plants within a single grow experience virtually identical conditions of light, temperature and nutrition, this result appears to support the proposition that potency is closely linked to the inherent genetic make-up of *Cannabis* plants, with some varieties being considerably more potent than others. This may well be the case, and strong evidence is mounting that the selective breeding of new varieties or strains is the reason that more potent *Cannabis* can be found now than in the past [33]. However, a factor which blurs the significance of this result is that as the four monoecious plants had all begun to produce fruit, their resin production would have levelled off and was probably in decline at the time of harvest [22,34].

In an ongoing internal (ESR) research project in which samples of New Zealand-grown *Cannabis* from hydroponics operations, outdoor cultivations and indoor soil operations are being tested, Hassan and Somerville have found THC values ranging from 4.2 to 18.1%, with an average of 10.9% ($n = 43$). While this work is still preliminary in nature, it contrasts strongly with the THC survey of New Zealand *Cannabis* reported by Poulsen and Sutherland [23], in which the female heads tested ranged from 1.3 to 9.7% THC, and 85% of all samples had potencies between 1 and 5%. These workers concluded that there had been no significant increase in the

**Table 6**
STR genotypes of Grow 1 plant samples.

| | ANUC301 | | ANUC302 | | ANUC303 | | ANUC304 | | ANUC306 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Plant 1 | 225 | 225 | 152 | 152 | 145 | 152 | 139 | 204 | 75 | 88 |
| Plant 2 | 225 | 225 | 138 | 155 | 142 | 145 | 139 | 139 | 75 | 88 |
| Plant 3 | 225 | 243 | 152 | 152 | 145 | 152 | 139 | 204 | 75 | 88 |
| Plant 4 | 225 | 243 | 152 | 152 | 145 | 152 | 139 | 204 | 75 | 88 |
| Plant 5 | 225 | 243 | 138 | 155 | 142 | 145 | 139 | 139 | 75 | 88 |
| Plant 6 | 225 | 225 | 138 | 152 | 155 | 142 | 145 | 152 | 139 | 204 | 75 | 88 |

**Table 7**
STR genotypes of Grow 3 plant samples.

| | ANUC301 | | ANUC302 | | ANUC303 | | ANUC304 | | ANUC306 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Plant 1 | 225 | 225 | 138 | 152 | 145 | 152 | 189 | 189 | 81 | 88 |
| Plant 2 | 225 | 225 | 138 | 152 | 145 | 152 | 189 | 189 | 81 | 88 |
| Plant 3 | 225 | 225 | 138 | 152 | 145 | 152 | 189 | 189 | 81 | 88 |
| Plant 4 | 225 | 225 | 138 | 152 | 145 | 152 | 189 | 189 | 81 | 88 |
| Plant 5 | 225 | 225 | 138 | 152 | 145 | 152 | 189 | 189 | 81 | 88 |
| Plant 6 | 225 | 225 | 138 | 152 | 145 | 152 | 189 | 189 | 81 | 88 |

average potency of *Cannabis* leaf or flowering head between 1976 and 1996. In light of the results of Operation REAP, and Hassan and Somerville's study however, it now appears clear that in the period since 1996, the potency of *Cannabis* grown in New Zealand has dramatically increased.

### 3.5. DNA analysis

DNA was successfully extracted from the dried leaf material of the 12 plant samples provided (from Grow 1 and Grow 3). For each extract, approximately 10 ng of DNA was amplified using the five trinucleotide STR markers described in Section 2.4, following Gilmore et al. [24]. Tables 6 and 7 summarise the genotypes observed after capillary electrophoresis and data analysis. The allelic designation is based on the observed size of each fragment in base pairs, as determined using GeneScan software.

All of the plants in Grow 3 were found to share the same genetic profile (Table 7). This finding is what would be expected if these plants were clones from the same parent stock.

Grow 1 plants exhibited a greater level of genetic diversity, with up to three different alleles seen at two of the loci (ANUC302 and ANUC303). A single plant (plant 6) exhibited a unique profile with three alleles seen at two of the loci (ANUC302 and ANUC303). This genotype was reproducibly obtained from multiple samples of plant 6 leaf material, with those triallelic loci exhibiting peaks of approximately equal height. These observations along with the absence of additional peaks at the remaining loci support the conclusion that this profile is not due to cross-contamination.

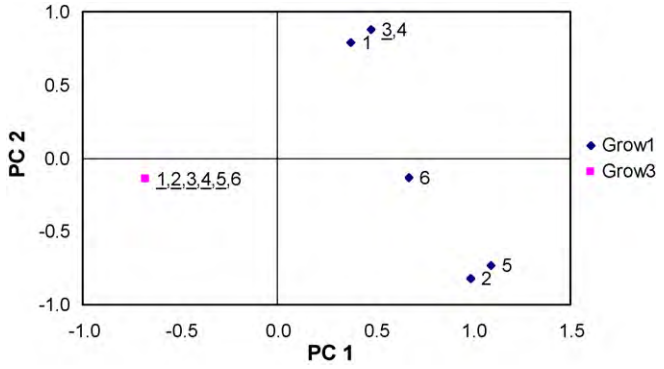


**Fig. 16.** Graph depicting the relationship between the first two principal coordinates obtained from principal component analysis of the Grow 1 and Grow 3 populations. Underlined numbers indicate plants sharing the same point on the graph.

The triallelic pattern is suggestive of polyploidy, where multiple copies of the genome are present, a situation common in plants with no adverse effects. Whilst *Cannabis* is largely diploid, genotypes have previously been reported exhibiting up to four alleles at a single locus [35], suggestive of polyploidy. Other explanations of multi-allele patterns include locus duplication and aneuploidy.

Analysis of DNA profiling results using Principal Coordinate Analysis allowed visualisation of the genetic relationship both within and between the plants in the two grows. Fig. 16 illustrates how the Grow 1 plants can be separated into genetically distinct groups that are themselves separate from the single genetic entity represented by the Grow 3 population.

It is acknowledged that the limited number of markers used on the small number of plants in this study provent any clear conclusions being drawn. However, the three groups seen in Grow 1 are consistent with the plants having originated from two distinct genetic origins, with plant 6 sharing alleles with both of the other two groups. The genetic grouping is aligned with the phenotypic and chemical differences observed (plants 1, 3, and 4 being monoecious, indica-like, with lower THC levels and plants 2 and 5 being dioecious, sativa-like, with higher THC levels). While plant 6 shared alleles with both groups in Grow 1, this plant was phenotypically and chemically grouped with plants 1, 3, and 4. All Grow 1 genotypes are clearly genetically separate and distinct from the Grow 3 plants.

## 4. Conclusion

The aim of this study was to ascertain the potential yield of *Cannabis* female flowering head which could be obtained from crops of six plants grown in a given area using a particular growing method (ScrOG). This information is needed by the New Zealand Courts when they are considering the severity of the offending, and in particular, how much income is being derived from such an illegal operation.

As an adjunct to the study, we made phenotypic observations, analysed the THC levels from the plants grown and undertook DNA analysis in order to see whether there was any genetic basis to the chemical and phenotypic differences observed.

Of the 18 plants grown, the yield varied between a maximum of 47.27 oz and 11.99 oz of dry, groomed female flowering head per

plant, with an average of just over 1.5 lb of head per plant. This demonstrates that it is possible to obtain at least 47.27 oz from a single plant. However, it has not been possible to show whether greater yields are possible, or to determine the mean or variance to be expected from such a method. This is mainly for two reasons: (1) the problems encountered during the growing, and (2) the small number of plants grown in this study.

This study has demonstrated that it is possible to obtain THC levels up to a maximum of 30% from *Cannabis* plants available in New Zealand. However, further research is needed to ascertain what the mean and variance of THC levels in New Zealand *Cannabis* populations may be, and the work of Hassan and Sommerville, referred to in Section 3.4, is intending to address this question.

DNA analysis distinguished distinct groups of plants in general agreement with the phenotypic variation observed, with the exception of one plant of the *indica*-like group from Grow 1. For example, DNA analysis determined that the two plants that exhibited the characteristics of the subspecies *sativa* were closely related. These two plants produced significantly higher THC levels than the four plants with the physical characteristics of the subspecies *indica*. As all other environmental and nutritional parameters affecting the six plants were controlled, this suggests that plant variety influences THC levels. A much wider study would be required however, to ascertain whether varieties within these two subspecies consistently produce THC levels within distinct ranges, or whether there is also considerable variation in THC levels within these subspecies.

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