MANAGEMENT OF Δ^9 THC LEVELS IN INDUSTRIAL HEMP GROWN FOR FIBRE AND GRAIN IN ONTARIO CANADA, 1995-1999.

By

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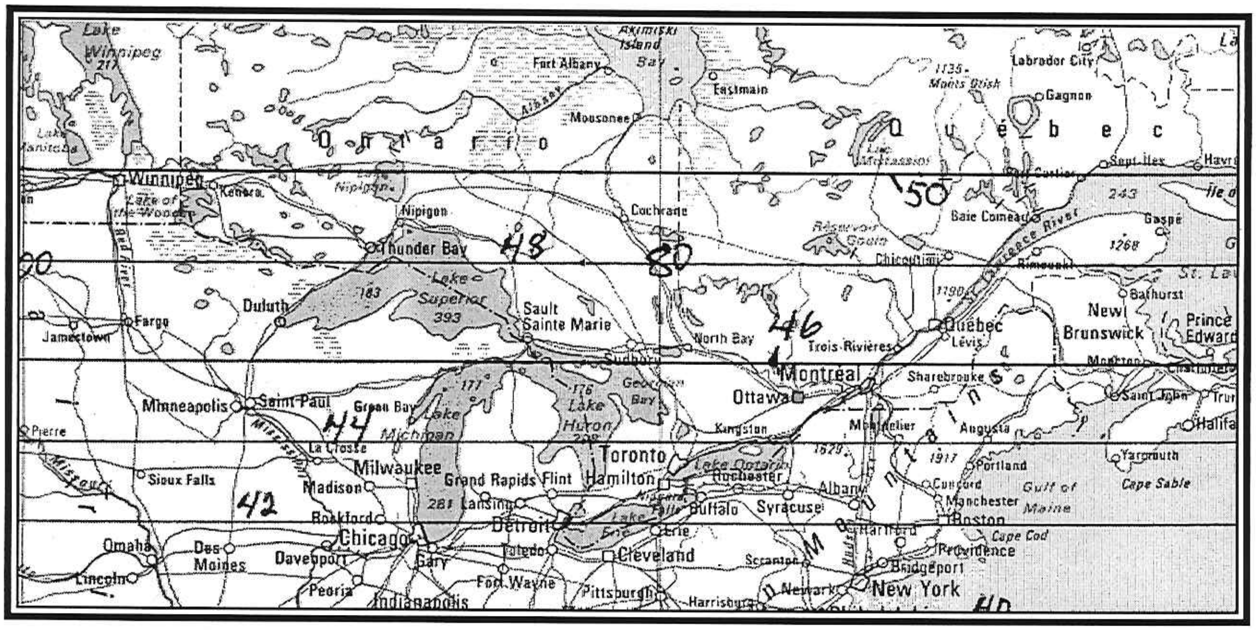


INTRODUCTION

After over 60 years of absence, industrial hemp has reappeared into the North American agriculture scene. The former Honorable Minister of Agriculture, Food and Rural Affairs for the province of Ontario, Canada, Noble Villeneuve, described the reappearance of industrial hemp as an agricultural "*Rip Van Winkle*". As *Rip Van Winkle* woke up to a whole new generation in his home village, so also industrial hemp in its renaissance in North America is emerging to an entire new age of THC (Δ^9 tetrahydrocannabinol) paranoia, politics, technology and markets. Paranoia surrounding the perceived dangers of Δ^9 THC took industrial hemp out of the North American agriculture scene during the late 1930's. Today we are still fighting the same war over the same issues, social concerns and politics of cultivating *Cannabis sativa* as industrial hemp. The issues are primarily fears and political pressures surrounding Δ^9 THC as a psychoactive drug produced in varying levels (<1%) by industrial hemp. Δ^9 THC is part of a 21 carbon chemical family called *Cannabinoids*, only found in *Cannabis* plants. It is soluble in water (2.8 mg/ml at 23 degrees C) with a boiling point at 200 degrees C and a molecular weight of 314.47 (Italy, Russia). The glandular secretory system in *Cannabis sativa* consists of three types of capitate glandular hairs (bulbous, capitate-sessile and capitate stalked) distinguishable by their morphology, development and physiology (Hammond, Scheifele & Dragla 2000). These glands occur together in greatest abundance and developmental complexity on the abaxial surface of the bracts of the inflorescence. Bulbous and capitate-sessile glands are initiated on very young bract primordia and attain maturity during early stages of bract growth. The capitate stalked glands are initiated later in bract development and undergo development and maturation on medium to full sized bracts. On mature bracts (8-10 mm), capitate-stalked glands with tall multi-cellular stalks are scattered over most of the bract along with the mature capitate-sessile and bulbous glands. Capitate-stalked gland are usually absent along the bract margins.

The exudate of these glandular cells – containing Cannabinoids, especially Δ^9 THC accumulates between the cuticle and the membranes of the cells. The exudate is a sticky, brown liquid, with a specific sharp smell. The glands ooze several volatile compounds such as terpenes, ketones and esters which produce the characteristic fragrant sweet "marijuana" odor", very prominent in the proximity of any industrial hemp field. The production and secretion of Cannabinoids in Cannabis plants is a hereditary genetic (closely linked independent genes (Sytnik)) characteristic, strongly influenced by environment (Bocsa, Scheifele). The highest concentration of secretory glands is found in young leaves, bracts and paired pistils, especially in the inflorescence of pistillate flowering plants. The Δ^9 THC levels in the bracts are 4 to 5 times those in the leaves (Hemphill).

The biggest threat to a viable industrial hemp crop and industry in Canada and the United States is the social and political fear surrounding the effects of Δ^9 THC, even though present in varying levels less than one percent.

Health Canada (HC) established the maximum levels for Δ^9 THC in commercial cultivated industrial hemp crops as 0.3% in the leaves and flowering heads in the top 1/3 of the plant (entire fruit-bearing part of the plant) when the seed begins to mature (when the first seeds of 50% of the plants are resistant to compression) at 50% pollen shed and raw and semi-processed products from grain as 10 ppm. The prospective industrial hemp producer must obtain a cultivation license to cultivate a minimum of 4 hectares of commercial industrial hemp. The regulations require the producer to have the field or variety independently sampled at his cost at 50% pollen shedding time for Δ^9 THC analysis. The sampled tissue (leaves and flowering heads in top 1/3 of plant) must be analyzed for Δ^9 THC levels by a certified laboratory (Health Canada). A successful hemp industry in Canada and United States requires the diligent understanding and successful management of the Δ^9 THC levels both in the crop, raw grain and semi-processed products. This necessitates a thorough understanding of the genotype/environment interactions affecting Δ^9 THC levels in the growing plants, harvested grain and resulting processed grain products.

The authors will discuss the Δ^9 THC analyzed data for Ontario from 1995 to 1999 from commercial and research grown industrial hemp crops over a wide range of soil, climate and genotypes from the 42nd to 49th latitude (a north/south distance of 870 km) and 80th to 94th longitude (a east/west distance of 1080 km). See map on page 1.

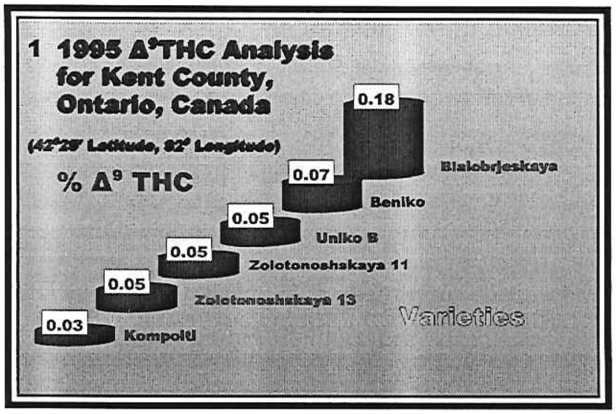
ACKNOWLEDGMENTS AND FUNDING

The authors wish to acknowledge the significant efforts of all the research technical staff at the respective research stations across northern Ontario for successfully conducting the industrial hemp trials for fibre and grain production. Also, the support from all the participating industrial hemp growers across northern Ontario must be acknowledged.

Funding in support of the industrial hemp research and production in Ontario was from a variety of district development corporations (Kent County, Patricia area, Rainy River, Thunder Bay, Sudbury and Manitoulin Is.), Kenex Ltd., Ontario Tobacco Diversification Funding, Ontario Soil & Crop Improvement Association, Northern Ontario Heritage Fund Corporation, Dryden Federation of Agriculture, Industrial Research Assistance Program of the National Research Council, Can Adapt (Agriculture Adaptation Council) and Thunder Bay Hemp Grower's Association. In-Kind contributions were from Quetico Centre (Atikokan), Kenex Ltd., Emo Feed Service, Thunder Bay Co-op Farm Supply, Lakehead University, farmer co-operative industrial hemp growers, Kemptville College/University of Guelph, Ridgetown College/University of Guelph and The Ontario Ministry of Agriculture, Food and Rural Affairs.

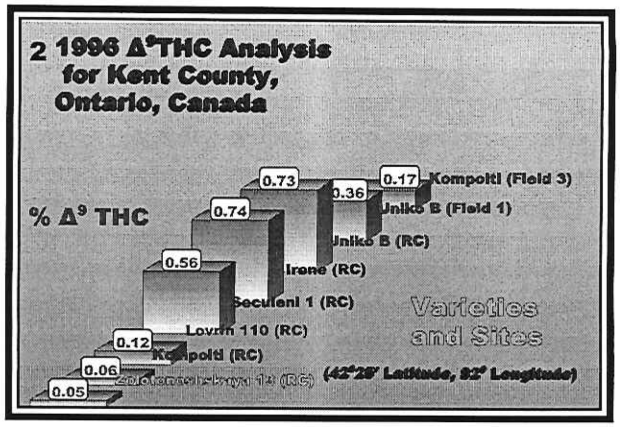
METHODOLOGY

Tissue samples were harvested by the authors from both commercial and research crops following the protocol outlined by Health Canada published regulations (Health Canada). The 1998 sampling of the two monoecious Romanian industrial hemp varieties, Secuieni 1 and Irene, at 50 days post emergence was an additional requirement stipulated in the licenses by Health Canada due to conditional approval of these two varieties to be grown in Canada. The grain samples for laboratory testing were dried to 4-5% moisture and thoroughly cleaned by air cleaning only unless otherwise stipulated. Oil samples for laboratory testing were gathered immediately after extraction (unfiltered). Meatherall Consulting, Winnipeg, Manitoba and Maxxam Analytics, Mississauga, Ontario did the Δ^9 THC analyses.

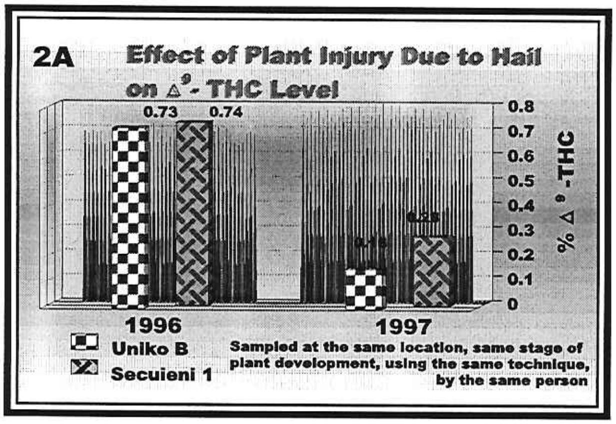


RESULTS AND DISCUSSION

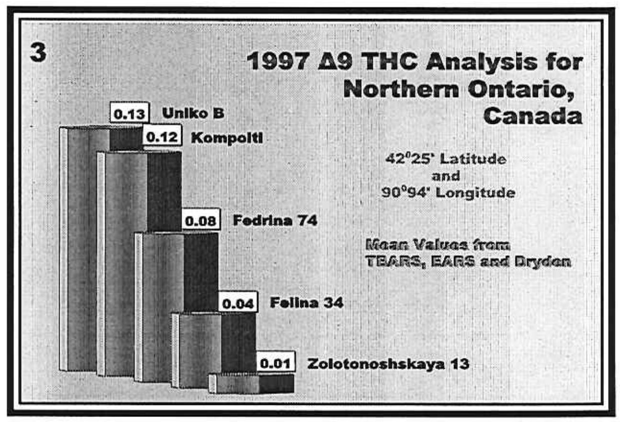
1995. The industrial hemp varieties evaluated in Ridgetown College (RCAT), Kent County in 1995 (42°25' latitude and 82° longitude) were Kompolti, Zolotonoshskaya (Zolo) 13, Zolo 11, Uniko B, Beniko and Bialobrzeskie (Scheifele & Pinsonneault 1995). The soil type was clay loam. The Δ^9 THC levels for these 6 varieties are summarized in ***Graph 1***. Note that all varieties except Bialobrzeskie (0.18% but still <0.3%) had very low levels between 0.03 and 0.07%.

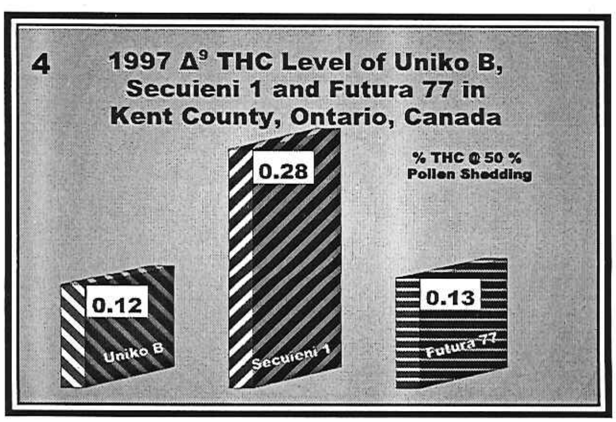


1996. The six industrial hemp varieties evaluated at RCAT (RC) in Kent County in 1996 (Scheifele & Dragla 1997) were Secuieni 1, Irene, Lovrin 110, Zolo 13, Kompolti and Unico B. The Δ^9 THC levels are summarized in ***Graph 2.*** The soil type was again clay loam. Kompolti and Zolotonoshskaya 13 had values between 0.05 and 0.06% at RCAT, similar to 1995. However, Kompolti in field 3 had a level of 0.17%, 3 times higher than at RCAT. Secuieni 1, Unico B and Irene had very high levels at RCAT, 0.56, 0.73 and 0.74% respectively; Unico B had a level of 0.36% in field 1. Severe weather, excessive rain resulting in flooding shortly after emergence, a devastating hailstorm 5 weeks after seeding and a very droughty August, contributed to the stresses resulting in these high levels of Δ^9 THC. The persistent low levels in Kompolti and Zolo 13 at RCAT in 1995 and 1996 cannot be explained. **Graph 2A** demonstrates the effect of the hail injury on Δ^9 THC levels of Unico B and Secuieni 1 (0.73% and 0.74% respectively) at RCAT in 1996 compared to no hail injury at the same location in 1997 (0.15% and 0.28% respectively).



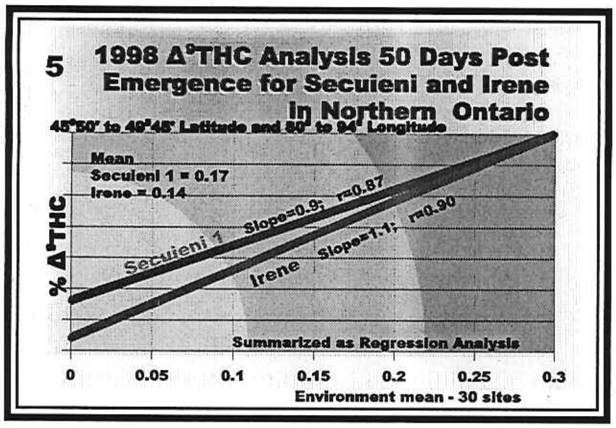
1997. ***Graph 3*** summarizes the Δ^9 THC levels for 5 industrial hemp varieties evaluated in 3 research sites in northern Ontario in 1997 (48°30' to 49°45' latitude and 90° to 94° longitude) (Scheifele 1998/1997). Zolo 13 had a 0.01% Δ^9 THC level, Felina 34 and Fedrina 74 were 0.04 and 0.08% respectively. Kompolti and Unico B were both 0.12 and 0.13% respectively. There was no significant variation observed between the 3 research sites. TBARS was sandy loam soil, EARS and Dryden were clay loam soil.





Graph 4 summarizes the Δ^9 THC levels from Kent County (42^0 25' latitude) for Unico B, Secuieni 1 and Futura 77 in 1997 (0.12%, 0.28% and 0.13% respectively) (Dragla & Laprise 1997). In 1997 Unico B demonstrated very little difference in Δ^9 THC levels from Kent county at 42^0 25' latitude to northern Ontario at 48°30' to 49^0 45' latitude.

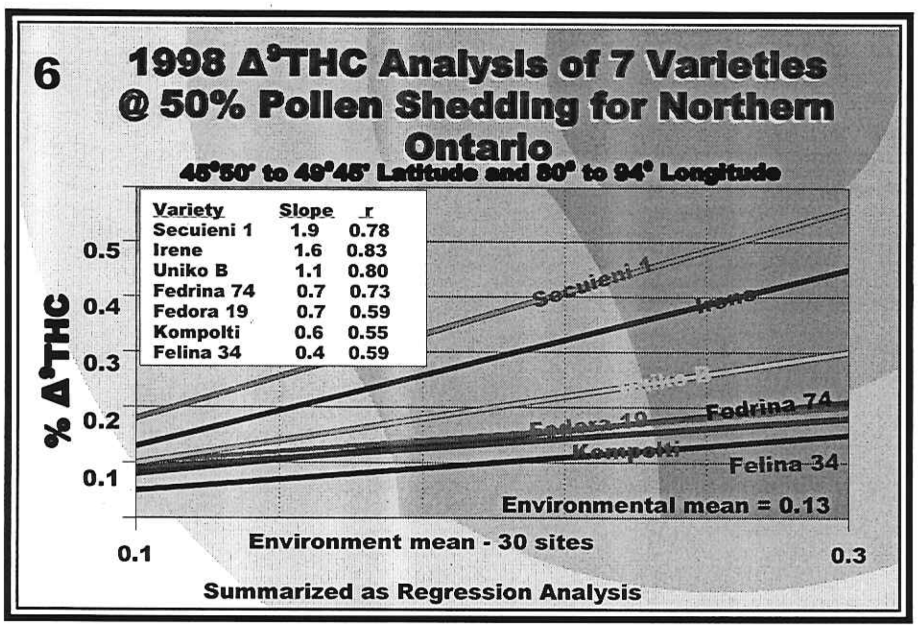
1998. The Δ^9 THC results for Secuieni 1 and Irene across 30 sites in Northern Ontario at 50 days post emergence are summarized in **Graph 5** in a regression analysis summary (Scheifele 1998, Scheifele et al.1999). The regression analysis of the individual levels



regressed over the respective environmental mean for the site gave a relatively strong correlation of 87% and 90% for Secuieni 1 and Irene respectively.

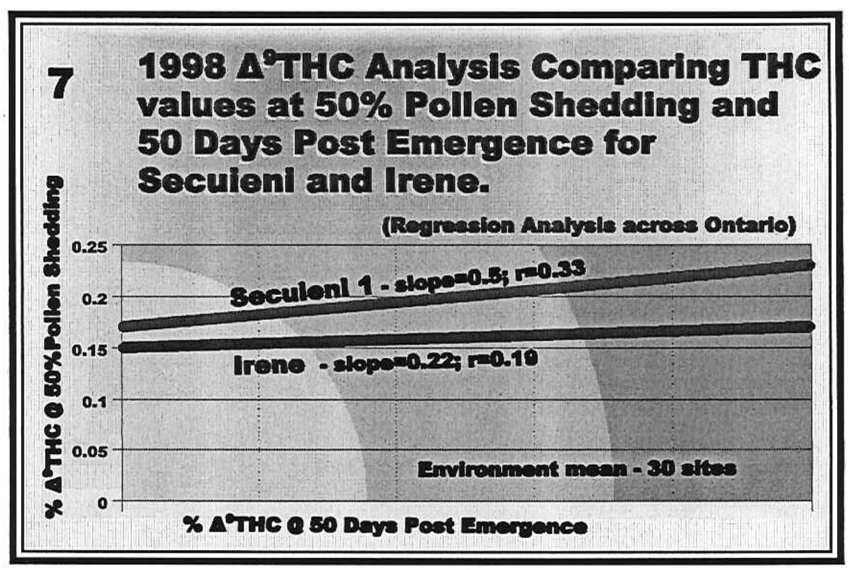
Both responded similarly with the environment with slopes of 0.9 and 1.1 respectively. The mean values for Secuieni 1 and Irene were 0.17% and 0.14% respectively. Both Secuieni 1 and Irene demonstrated relatively strong genetic stability as indicated by the relatively high R^2 values which were 76% and 81% respectively.

Graph **6** summarizes the regression analysis for seven varieties including Secuieni 1 and Irene sampled at 50% pollen shedding (about 30 days later). Note that the responses of both Secuieni 1 and Irene to the environments (slopes) increased substantially to 1.9 and 1.6 respectively. The mean level value for Secuieni 1 increased to 0.23% from 0.17% at 50 days post emergence. The correlation of the Δ^9 THC values to the environment was still relatively strong at 78% and 83% (R^2 = 61% and 69% respectively) for Secuieni 1 and Irene. Both Secuieni 1 and Irene demonstrated consistently higher Δ^9 THC levels than any of the other 5 varieties. On several occasions both Secuieni 1 and Irene exceeded the 0.3% level.

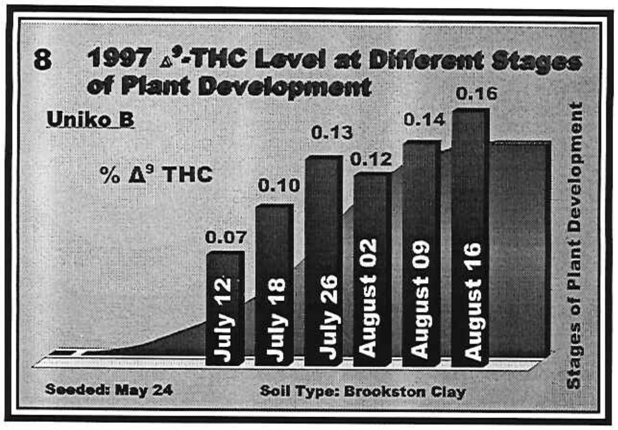


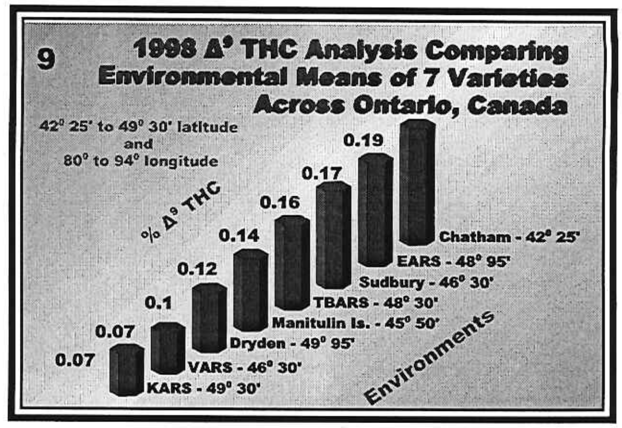
Graph 7 summarizes the correlation of Δ^9 THC at 50 days post emergence to 50% pollen shedding for Secuieni 1 and Irene. Note that the correlation values of Secuieni 1 and Irene (0.33% and 0.19% respectively) are very poor. Prediction of Δ^9 THC at 50 days post emergence for mature levels of Δ^9 THC is not possible.

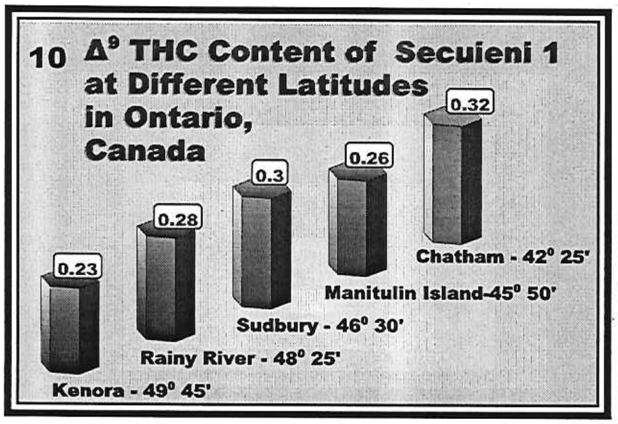
Graph 8 demonstrates the pattern of increasing Δ^9 THC levels in Unico B as the plant matures (Dragla 1997). Levels at 49 days post seeding are 0.07%, 55 days = 0.10%, 63 days = 0.13%, 70 days = 0.12%, 77 days = 0.14% and 84 days = 0.16%. As noted above the Δ^9 THC levels at 50% pollen shedding cannot be predicted from levels at 50 days post emergence. It can only be predicted that levels will be higher.



The regression analysis summarized in **Graph 6** demonstrates the genetic stability of Unico B, Secuieni 1 and Irene







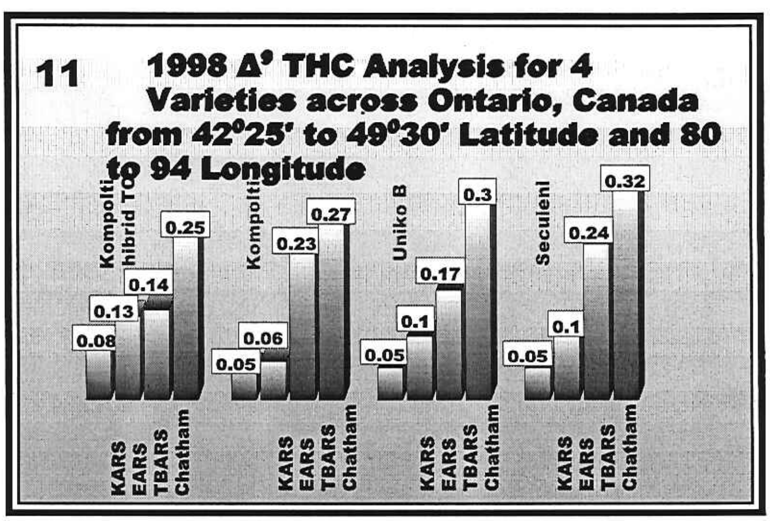
with relatively strong coefficient values (R^2 = 64, 61 and 69% respectively). Felina 34, Fedrina 74, Kompolti and Fedora 19 demonstrated much less genetic stability (R^2 = 35, 53, 30 and 35% respectively), lower mean Δ^9 THC levels (0.08, 0.08, 0.09 and 0.11% respectively) and less response to the environment (slopes = 0.4, 0.7, 0.6, 0.65 respectively).

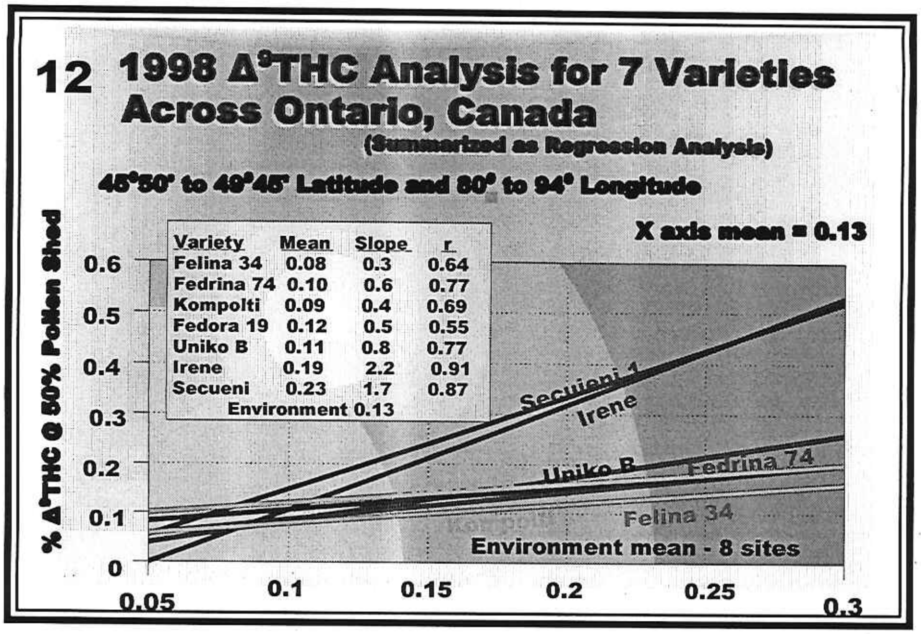
Graph 9 demonstrates the increase in mean levels of Δ^9 THC for 7 varieties (Felina 34, Fedrina 74, Kompolti, Fedora 19, Unico B, Irene and Secuieni 1) across Ontario from the lowest latitude of 42°25' (0.19%) to highest latitude of 49°30' (0.07%) (Scheifele et al. 1999, Dragla 1998). ***Graph 10*** demonstrates the similar increase in Δ^9 THC levels for Secuieni 1 from the highest latitude of 49°45' (0.23%) to the lowest latitude of 42°25' (0.32%) (Scheifele et al. 1999).

The data summarized in ***Graph 11*** demonstrates the Δ^9 THC variability due to environments and latitude for Kompolti Hybrid TC, Kompolti, Unico B and Secuieni 1 (Scheifele et al. 1999). Kompolti Hybrid TC, Unico B and Secuieni 1 showed the greatest response for increased *delta 9* THC levels as latitude decreased (Chatham being the lowest latitude of 42°25'). KARS was the site with the highest latitude (49°30').

Graph 12 summarizes the Δ^9 THC data at 50% mid pollen shedding for 7 varieties across 8 sites in Ontario (42°25' to 49°30' latitude and 80° to 94° longitude) in a regression analysis form. These data again demonstrate the relative strong genetic stability of Irene and Secuieni 1 (R^2 = 83% and 76% respectively) and strong environmental response (slope = 2.2 and 1.7 respectively) with relative high mean level Δ^9 THC values of 0.19% and 0.23% respectively. Felina 34, Fedrina 74, Kompolti, Fedora 19 and Unico B demonstrate similar characteristics as discussed for **Graph 6**. Unico B demonstrated less genetic stability across all of Ontario than just northern Ontario (higher latitude).

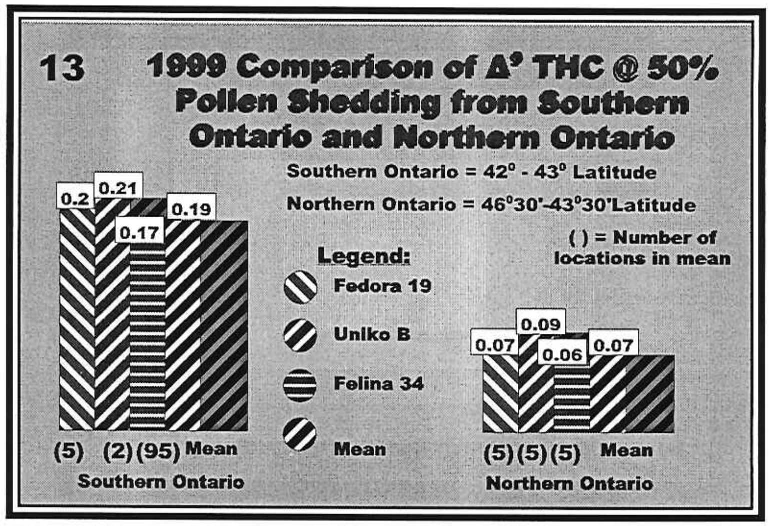
1999. The 1999 data comparing Δ^9 THC levels from southern Ontario to northern Ontario is summarized in **Graph 13** for Fedora 19, Unico B and Felina 34 (Scheifele & Dragla 1999, Scheifele 1999). The ( ) number represents the number of sites averaged in the mean. The southern Ontario *delta 9* THC values are again consistently higher than those of northern Ontario demonstrating a latitude effect.



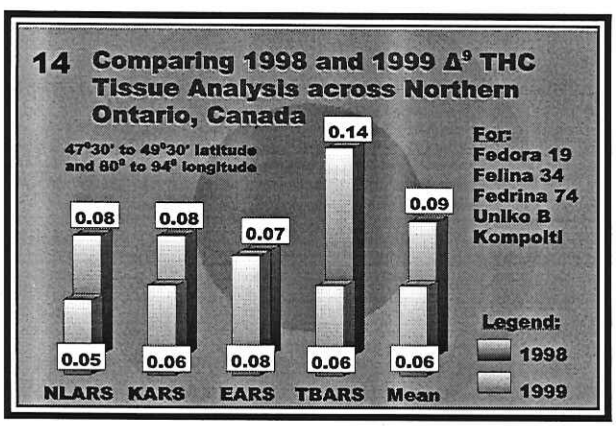


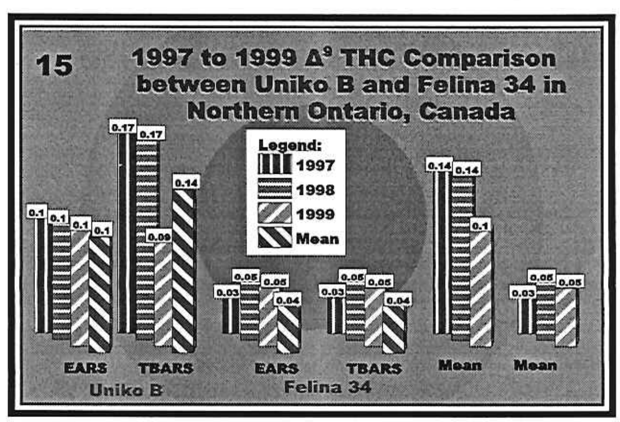
1998-1999. Graph 14 compares the 1998 Δ^9 THC data for 5 varieties (Fedora 19, Felina 34, Fedrina 74, Uniko B and Kompolti) across 4 northern Ontario research sites to 1999 data. TBARS had significantly higher Δ^9 THC levels in 1998 (0.14%, sandy loam soil) than in 1999 (clay loam) and the other 3 research sites (clay loam). The 1998 Δ^9 THC levels were consistently higher (0.09% compared to 0.06%) for all 5 sites than in 1999.

1997-1999. Graph 15 compares the Δ^9 THC levels at 50% pollen shedding for Unico B and Felina 34 across 2 northern Ontario research stations (TBARS and Emo Agricultural Research Station (EARS)) from 1997 to 1999. Unico B had significantly higher levels of Δ^9 THC at TBARS in 1997 (0.17%) and 1998 (0.17%) (sandy loam soil) compared to 1999 (0.09%) (clay loam soil). Felina 34 was very consistent for year and site and ranged from 0.03% to 0.05%. Unico B was consistently higher for Δ^9 THC than Felina 34 for year and site (mean = 0.14, 0.14 and 0.10% for 1997,1998 and 1999 respectively compared to Felina 34 which was 0.03, 0.05 and 0.05 respectively). **Graph 16** compares Δ^9 THC levels for the mean of 5 varieties (Fedora 19, Felina 34,

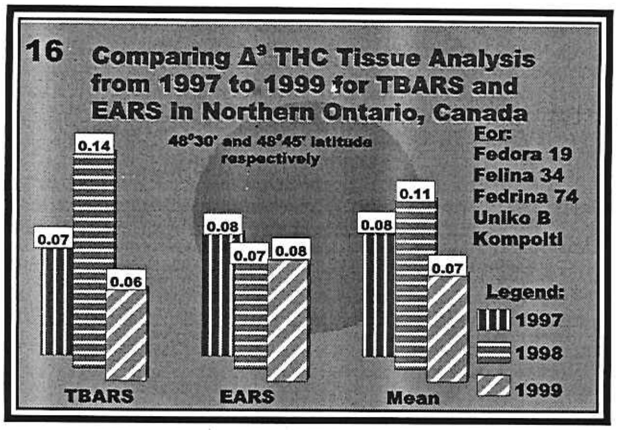


Fedrina 74, Unico B and Kompolti) at TBARS (sandy loam in 1997 & 1998 and clay loam in 1999) and EARS (clay loam) in northern Ontario from 1997 to 1999. TBARS demonstrated significantly higher Δ^9 THC levels in 1998.



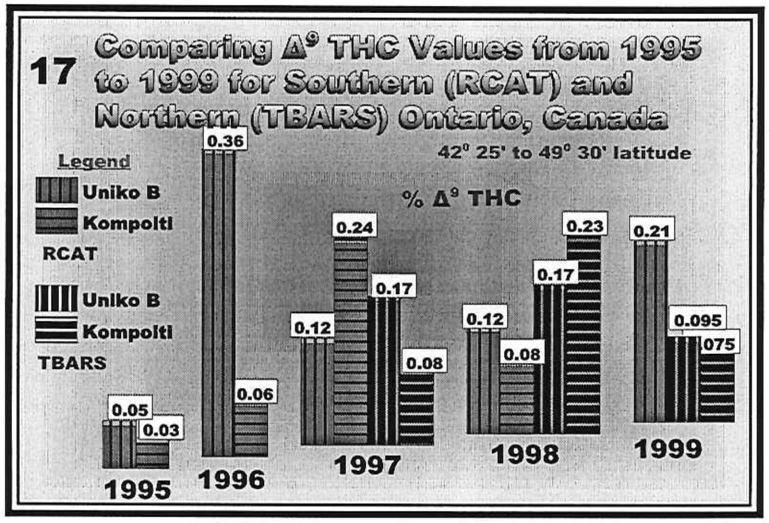


1995-1999. Graph 17 summarizes the Δ^9 THC levels for Unico B and Kompolti for southern (1997 to 1999) and northern Ontario (1995 to 1999). Unico B demonstrated a range of Δ^9 THC levels from 0.05% to



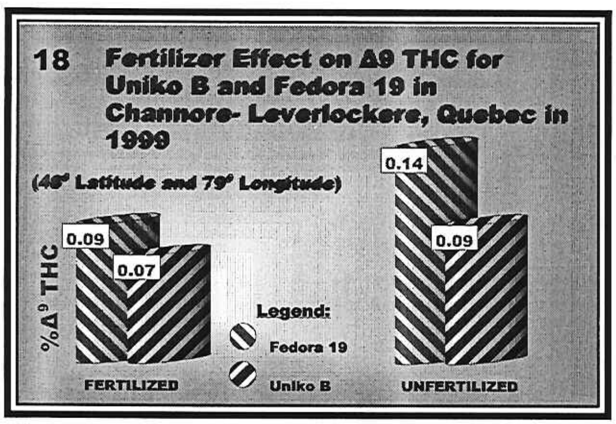
0.36% at RCAT and 0.10% to 0.17% at TBARS. Kompolti demonstrated a range from 0.03% to 0.24% at RCAT and 0.08% to 0.23% at TBARS. Both varieties demonstrated considerable variability in the range of Δ^9 THC levels from year to year at both extreme locations.

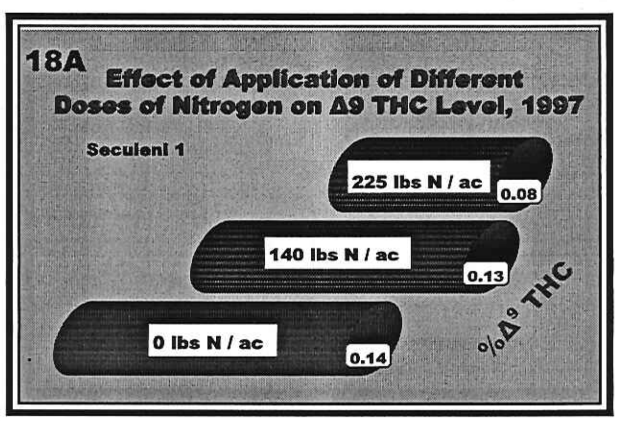
Effect of Fertility and Soil Types on Δ^9 THC Levels. **Graph 18** summarizes data for Fedora 19 and Unico B from Channore-Leverlochere, Quebec (48° latitude and 79° longitude) demonstrating the stress effect on Δ^9 THC levels from lack of adequate fertility (Scheifele 1999). The unfertilized plots were consistently higher in Δ^9 THC level. Fedora 19 was 0.09% (fertilized) compared to 0.14% (un-fertilized) and Unico B was 0.07% compared to 0.9% respectively. **Graph 18A** (Dragla 1997) demonstrates data from 1997 at RCAT comparing Δ^9 THC levels for Secuieni 1 at zero nitrogen to 202.5 kg/ha (0.14% to 0.08% *delta 9* THC respectively). The stress of lack of nitrogen or poor fertility can significantly cause the industrial hemp plant to produce higher Δ^9 THC levels. **Graph 18B** (Dragla 1997) demonstrates the effect of different soil types in Essex and Kent Counties in 1997 on Δ^9 THC levels of Unico B. Brookston clay soil type produced the highest levels of Δ^9 THC (0.16%). **Graph 18C** (Dragla 1997) demonstrates the impact of clyde loam soil type to Brookston clay soil types on Δ^9 THC levels for Unico B, Secuieni 1 and Futura. Secuieni 1 had significantly higher levels (0.30%) on Clyde loam compared to Brookston clay (0.16%). **Graph 18D** (Dragla 1998) demonstrates the Δ^9 THC levels for Fedrina 74 averaged over 4 commercial fields on each soil type in 1998. Clay soil produced the higher levels (0.21%) and muck soils the lowest (0.15%).

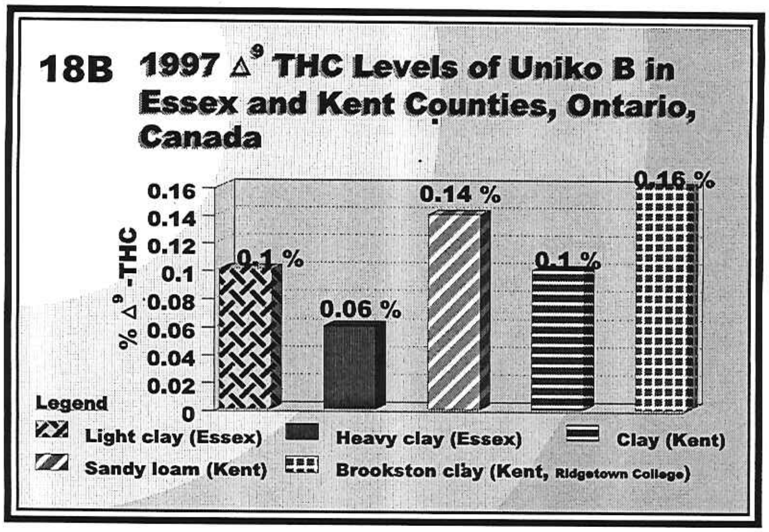


Relationship of Δ^9 THC Levels in Tissue of Inflorescence Compared to After Harvest in Grain and Extracted Oil. **Graph 19** summarizes the data for Fasamo, Fedora 19 and Felina 34 comparing Δ^9 THC levels for grain and oil compared to the Δ^9 THC levels of the tissue from plant tops of the same plots as harvested grain at 50% mid pollen shedding (Scheifele 1999). The mean Δ^9 THC levels for the oil and grain were 20.7 ppm and 5.03 ppm respectively compared to 0.05%, 0.01% and 0.05% for tops at 50% pollen shedding from Fasamo, Fedora 19 and Felina 34 respectively.

The mean Δ^9 THC level for oil was about 4 times the mean level of the grain. Even though all three varieties had very low Δ^9 THC levels in the plant tissue (50% mid pollen shed) the harvested grain still had consistently

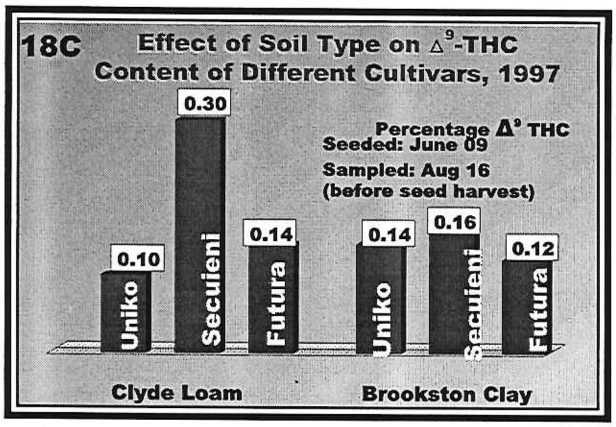


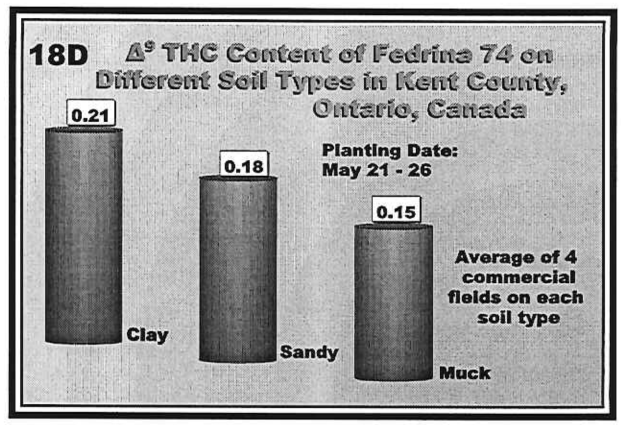




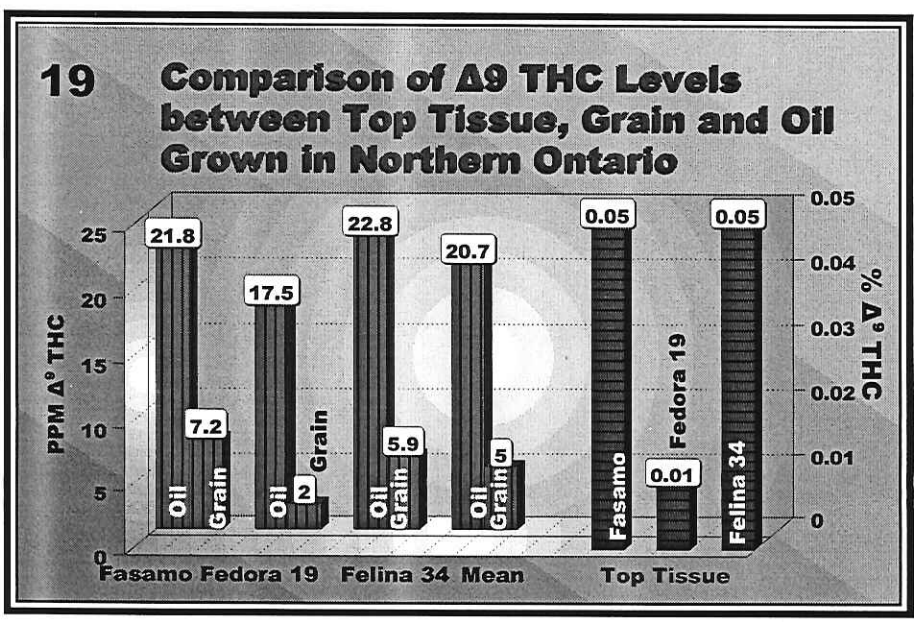
very high levels of Δ^9 THC levels (mean of 5.03 ppm) and the resulting extracted oil had a mean level of 20.7 ppm (maximum regulation limit is 10 ppm). The grain was properly and thoroughly air cleaned before oil extraction. Low Δ^9 THC levels in varieties in the field will not necessarily insure low levels on the harvested air cleaned grain before extraction. The industrial hemp seed contains zero Δ^9 THC levels (Bocsa & Mathe, Hemphill, Leson).

Comparison of Δ^9 THC THC Levels in Extracted Oil from Unwashed to Washed Seed. Graph 20 summarizes the data comparing Δ^9 THC levels for grain and oil from cold water rinsed seed



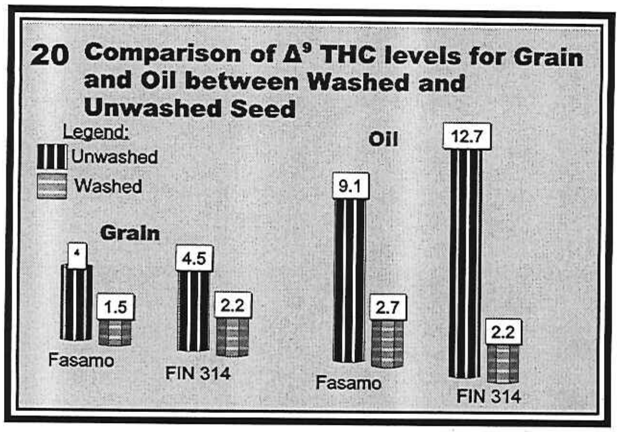


and non-rinsed seed (Scheifele 1999). Cold water rinsing followed by air or centrifuge drying the grain prior to oil extraction resulted in a reduction of Δ^9 THC levels in Fasamo and FIN 314 from 4.0 ppm to 1.5 ppm and 4.5 ppm to 2.2 ppm respectively. The resulting extracted oil was 9.1 ppm to 7.7 ppm and 12.7 ppm to 2.2 ppm respectively for Fasamo and FIN 314. The low Δ^9 THC level in the rinsed oil of FIN 314 (2.2 ppm) extracted from the grain with 2.2-ppm Δ^9 THC is unexplainable.

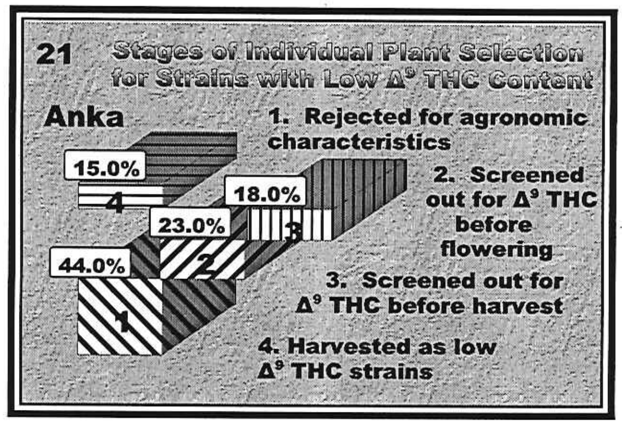


Δ^9 THC Management in Industrial Hemp Breeding.

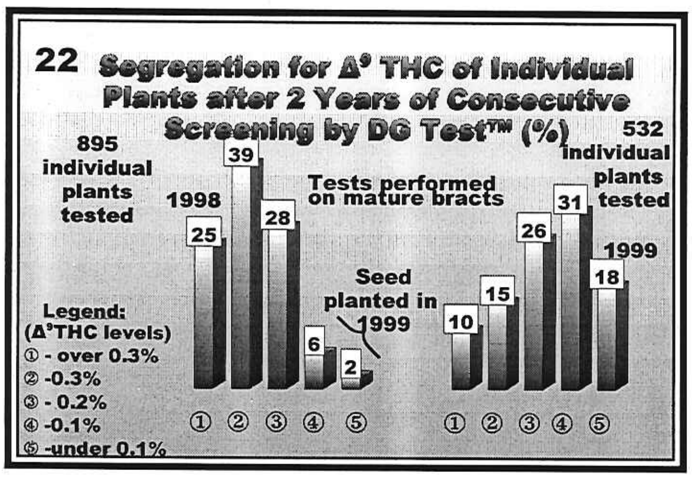
The breeding for low Δ^9 THC industrial hemp varieties (<0.3%) traditionally involves very labor intensive and costly screening of early generation breeding materials. This requires the chemical analysis in the laboratory of thousands of plants. Industrial Hemp Seed Development Company (IHSDC), Chatham, Ontario, Canada



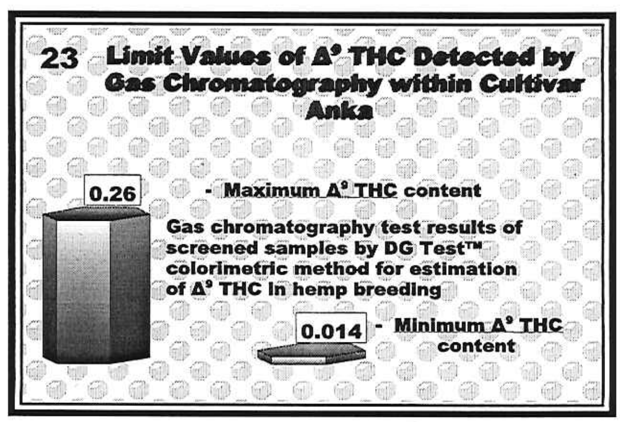
has developed a rapid, highly sensitive, and inexpensive quick test for in the field evaluation of thousands of plants. The test, called DG Test (Dragla 1999, 1999A & 1999B), only requires 2 minutes to develop a reaction for reading allowing for immediate plant selection in the field. Dragla should be directly contacted for more information concerning this "Δ^9 THC quick test". The following process (**Graph 21**) is recommended for rapid successful



selection of low THC genotypes in a breeding program. 1) The breeding selection site should be located at the lowest latitude available for maximum THC expression by the plant. 2) Select the plants exhibiting the desired agronomic characteristics. 3) Screen the selected plants for desired agronomic characteristics for low THC (using DG Test) before flowering. Test tissue from tops of plants. Destroy the high THC plants. 4) Re-screen plants before harvest (test on bracts) for selection of lowest THC genotypes. 5) Harvest the lowest testing



THC genotypes (<0.2%). IHSDC has been successful in applying this method selecting stable low THC genotypes at levels <0.05% Δ^9 THC. **Graph 22** demonstrates the potential progress made within one generation of selection in the variety, Anka. The distribution represents the Δ^9 THC profile from 895 individual plants screened in 1998 and only planting seed from group 4 and 5 (8% of the plants with <0.1%) in 1999. In 1999, only the plants from group 4 and 5 (49% of the plants with <0.1%)were saved for seed for the next generation. **Graph 23** compares the 1999 results from the DG Test to gas chromatography. Twenty-five plants individually tested using the DG Test with levels between 0.2%-0.3% were grouped and tested collectively using gas chromatography resulting in 0.26%. Similarly 25 plants testing 0.1% and less using the DG Test



were groped and tested collectively using gas chromatography resulting in 0.014%. The gas chromatography test results verify the accuracy of the DG Test for selecting genotypes for low *delta 9* THC.

CONCLUSIONS

Secuieni 1 and Irene both demonstrated relatively strong genetic stability (high R^2 values) and responded strongly to the environment (represented by steep regression slopes). Data from the 1996 and 1998 production seasons demonstrated these varieties' genetic ability to exceed Δ^9 THC levels of 0.3% quite frequently given appropriate environmental conditions. Gauca, Industrial Hemp Breeder, Secuieni Research Station, Romania, reports that there are improved strains of Secuieni 1 with Δ^9 THC contents <0.15% and are commercially available since 1998 (Gauca). The 1998 data demonstrated no correlation between the Δ^9 THC levels of the plant tops sampled at 50 days post emergence and 50% pollen shedding. The sampling and Δ^9 THC determination at 50 days post emergence is not predictive of Δ^9 THC levels at 50% pollen shedding even though it is predictively higher as the plants mature. Varieties, Fedora 19, Felina 34, Fedrina 74, Fasamo, Zolo 13 and 11 and Kompolti had consistently lower Δ^9 THC levels, showed weaker response to the environment and greater genotype x environment interactions. Unico B demonstrated a relatively strong response to the environment and year to year effect. It can be expected to occasionally exceed the 0.3% legal limit of Δ^9 THC given the proper environmental conditions. Bocsa reports improved strains of Unico B with low Δ^9 THC levels which are commercially available (Bocsa).

The Δ^9 THC levels can be significantly elevated in the leaves and inflorescence of plant tops by environmental stresses such as hail damage soil type and climate. Stresses caused by management practices such as lack of or inadequate fertility can result in elevated Δ^9 THC levels in the plant tops. Heavy clay soils can also effect crops with higher Δ^9 THC levels. The higher Δ^9 THC levels at Thunder Bay on sandy loam soils in 1997 and 1998 is unexplainable.

Some varieties respond greater than others for reduced Δ^9 THC levels due to latitude increases. Kompolti Hybrid THC, Unico B and Secuieni 1 demonstrated the greatest response to latitude change for Δ^9 THC levels. Most of the Canadian industrial hemp production is north of the 48° latitude which along with genetically bred low Δ^9 THC varieties will naturally reduce potential delta 9 THC levels in the field. Screening in breeding programs for genotypes with low Δ^9 THC levels should be done at southern (lower) latitude sites allowing for maximum expression for Δ^9 THC levels (Scheifele & Dragla 2000).

Low Δ^9 THC levels from inflorescence sampled at 50% pollen shedding does not insure low Δ^9 THC levels in harvested grain or extracted oil. The bracts enveloping the seed are sites of maximum Δ^9 THC concentration (4 to 5 times higher than leaves of the inflorescence), fall weather conditions such as rain and THC exudate contamination at harvest time can result in higher levels of Δ^9 THC than tolerated. The Δ^9 THC level in extracted oil can be expected to increase by four fold compared to the levels on the grain. Management practices, such as proper combine adjustments, minimal handling of grain prior to cleaning and proper cleaning will all contribute to reduced grain contamination of Δ^9 THC. Additional cleaning of the grain is required to remove the excessive Δ^9 THC contamination from the hulls of the seed. The levels of Δ^9 THC on the grain must be < 2 ppm for safe levels in the extracted oil (<10 ppm). Cold water rinsing of the grain followed by air or centrifuge drying can be an effective management practice effective to reduce the level of Δ^9 THC contamination. The hemp seed itself does not contain Δ^9 THC. Hulling the grain will also remove most of the contamination providing the equipment is properly designed to avoid additional contamination of the de-hulled nuts during hull removal.

Rapid progress can be made selecting for low Δ^9 THC genotypes in conventional plant breeding programs using proper sampling and selection techniques. The field application of the DG Test colorimetric method allows for inexpensive, rapid, accurate testing of individual plants, selecting for seed only those with very low Δ^9 THC levels.

Even though industrial hemp varieties continue to have less and less Δ^9 THC levels inherently, it will take continued diligent grain production and processing management practices to insure governments and the consumer with a consistent very low Δ^9 THC food product (<10 ppm). Zero tolerance by any government will limit the market significantly for any industrial hemp food products as long as any genetic capability exists in the plant to produce Δ^9 THC.

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