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Mineral Nutrition of *Cannabis sativa* L.

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ABSTRACT

Forensic laboratories can be called to examine illicit *Cannabis* samples (marijuana) to identify their geographical origin. They can also be required to compare different seizures to establish whether they were drawn from the same original lot. The quantitative determination of selected organic components is one of the criteria currently used in such investigations. This study aimed at evaluating the inorganic element pattern of marijuana as a possible additional diagnostic tool. Four commercial cultivars of *Cannabis sativa* L. were grown in field experiments planned so that edaphic and climatic growth conditions varied slightly among the fields. The experimental design produced six populations. Population variability for the elements sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), manganese (Mn), iron (Fe), zinc (Zn), and molybdenum (Mo) accumulation in leaves (L) and inflorescences (FL) of female plants was determined. For each element, the analytical data for the L and FL belonging to the same sample were pooled to simulate the chemical profile of marijuana preparations (L+FL). Within every population frequent important differences in elemental concentrations between corresponding L and FL fractions were detected, chiefly for the elements Ca, Mg, Cu, Mn, Fe, Zn, and Mo. This suggests that: i) the mean composition of marijuana produced in a single field depends on the relative amounts of plant parts harvesters pick, and ii) whenever a small drug sample is examined, the analytical outcome will be influenced by the weight ratio of the different plant parts which happen to make up the sample itself. Whatever the fraction considered (L, FL, and L+FL), a narrow scattering

of data for all elements except Na was observed within each population, with RSD values generally well below 10%. When populations were compared for their elemental composition, many significant differences were found; for the mock drugs (L+FL fractions) they were most frequently determined by Ca followed by Mo, K=Fe, Zn, Mn, Cu=Na, and Mg in this order of decreasing frequency. Multi variate (discriminant) statistical analysis for product description was effective in separating the L, FL, and L+FL fractions of the six populations.

INTRODUCTION

Cannabis sativa L. is an annual, principally dioecious tall weed belonging to the family Cannabinaceae (Turner et al., 1980). Cultivated or wild grown in geographical areas extremely dissimilar for climate and soil type, this plant evolved into numerous strains having wide different morphological (Quimby et al., 1973) and biochemical (Turner et al., 1980) characteristics. Abandoned as a fiber crop, *Cannabis* is still important economically as a source of crude drug preparations, mainly marijuana and hashish. The first is a dry mixture where plant tissues predominate, while hashish is essentially made of the resin extruded from the pistillate flowers. For their effects on man both drugs are illegal in many countries. Being able to use chemical analysis: i) to assess the geographical origin of a confiscated drug sample, and ii) to prove that seizures at different places are in fact sub-samples of the same lot, is important in forensic work and in monitoring unlawful traffic.

Cannabis is unique in its capacity of synthesizing cannabinoids (Turner et al., 1980), a class of compounds where the psychoactive properties of the plant reside. Davis et al. (1963) were the first to develop the concept that the cannabinoid profile of marijuana is indicative of the drug's geographical origin and correlated it with climatic factors. Today legal laboratories routinely use the quantitation of specific cannabinoids for *Cannabis* drug characterization (De Faubert Maunder, 1976; Wheals and Smith, 1975; Brenneisen and Elsohly, 1988). Causes for confusion are abundant, however, in view of the influence of sex, plant genetics, plant part considered, plant maturity, soil type, ageing and storage conditions of harvested material on the cannabinoid profile (De Faubert Maunder, 1976; Turner et al., 1979; Small and Beckstead, 1973; Hemphill et al., 1980; Coffman and Gentner, 1975). For a more accurate fingerprinting of drug samples, the determination of classes of organic compounds other than cannabinoids has been suggested, i.e., hydrocarbons (Mobarak et al., 1974), terpenes/terpenoids/alkanes (Brenneisen and Elsohly, 1988; Novotny et al., 1976), and headspace volatiles (Hood and Barry, 1978).

In addition to the use of the organic composition of *Cannabis* in connection with the two forensic problems previously outlined, elemental (mainly metal)

composition of materials has also been evaluated for its possible diagnostic value. Studies along this line (Leddicotte et al., 1965; Schlesinger et al., 1965; Wijesekera et al.,1988; Henke, 1977) have been dealing mostly with natural drugs other than marijuana and appear rather inconclusive, being based on too few, badly documented samples, whose small size concur to prevent a statistically convincing interpretation of the data. On the other hand, laboratories lacking supranational sponsorship have no chance of disposing of drug lots of definite origin and large enough to be representative of whole productions. In a greenhouse experiment directed to testing the existence of a leaf-soil nutrient relationship, hence a foreseeable practicability of using inorganic analysis for drug recognition, Coffmann and Gentner (1975) grew one *Cannabis* variant in different soils under identical light/temperature conditions. Later, they studied the inorganic composition of *Cannabis* foliar tissues as a function of NPK soil levels (Coffman and Gentner, 1977). Several significant correlation between plant and soil composition were found, pointing in favor of the soundness of the premises. However, the authors rightly stressed the introductory character of their research and the complexity of the problem, certainly its multi-variate nature (a situation quite similar to that found when it is cannabinoids that are considered).

In this report, an investigation was conducted where four strains of *Cannabis* were field-grown extensively under slightly different environmental conditions to give six populations altogether. The objectives were i) to obtain an indication of the differential accumulation of metal nutrients between vegetative leaves and whole inflorescences of female plants, ii) to evaluate the (natural) variability of nutrient concentrations to be expected in *Cannabis*, and iii) to verify if the use of discriminant analysis in describing a *Cannabis* population by way of the whole set of elemental concentrations could lead to a picture distinctive of the population itself.

MATERIALS AND METHODS

Cultivars and Planting Sites

Four dioecious varieties of fiber type *Cannabis* were considered, Carmagnola (Italy), Fibrimon 56R (France), Uniko BF2 (Hungary), and Fibranova (Italy) whose seeds were obtained through commercial channels directly from the countries given in the brackets. Four homogeneous fields of one hectar each were chosen so that their climatic, textural, and chemical attributes did not differ significantly. Located in northern Italy at Este, Diamantina, Ostellato, and Anzola, their geographical position is given in Figure 1. The proximity of the sites make them influenced by the same temperate climate but each has a distinctive micro climatic regime; in fact, the Este field lays upland within an hilly area and faces North while, further South, the other sites are situated in the Po Valley plains at different distances from the sea, with Anzola the furthest and at the foot of the Appennines

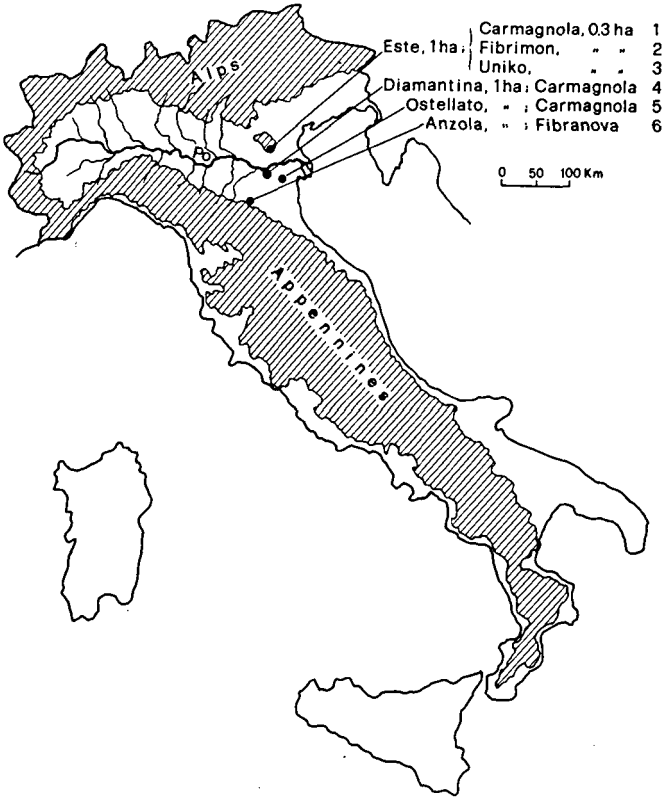

FIGURE 1. Map showing where *Cannabis* was grown. Reported are the size of the fields, the variety or varieties grown on each field and the identification number of the cultivations.

chain. Each field had been farmed as a unit in the preceding twenty years or more and had long undergone a corn, soybean, and sugarbeet rotation under correct NPK fertilization practices. Soils were sampled at the 0-40 cm depth according to a rectangular zig-zag model (Sabbe and Marx, 1987) to give composite samples for analysis.

Growth Conditions

Official regulatory constraints regarding *Cannabis* cultivation and seed market prevented a complete four (varieties) x four (soils) factorial experiment and only

the following combinations variety/soil were adopted: Carmagnola, Fibrimon, Uniko/Este (three varieties grown on the same field in adjacent plots of 0.33 ha each); Carmagnola/Diamantina; Carmagnola/Ostellato; Fibranova/Anzola (Figure 1). Thus six populations of *Cannabis* were produced numbered from 1 to 6 in the above given order (Figure 1). At the beginning of March, fertilizers were broadcast before disking at rates of 150 kg N, 40 kg P, and 83 kg K ha^{-1} and the application was not repeated. In early spring, all seed stocks were planted at 18 cm between seeds in the row with rows spaced 50 cm apart. Populations 1-3 (Este) amounted to at least 30,000 plants each and populations 4-6 three times as much.

Plant Sampling and Tissue Preparation

Harvest was restricted to female plants at the stage of maturity when their flowering tops had started developing seeds. Sampling was carried out by ideally dividing each cultivation into five equal parts along the rows and randomly collecting from every section ten plants which together formed one sample. Thus each population was described by five samples, singularly composed of ten individuals. The same day of harvest samples were processed. All vegetative leaves and the entire inflorescences were excised from the stalks (discarded) and kept separate to originate two fractions, hereafter referred to as L and FL, respectively (Figure 2). Unwashed plant tissues were oven dried at 85°C for 24 h and after removing the woody floral axes from the FL samples, all fractions were individually ground (60-mesh) and weighted. On the basis of the weights and the analytical data of L and FL of the same sample, the elemental composition of the material that would have been produced had the two fractions been mixed together, was calculated, thus mimicking the composition of marijuana. These virtual samples will be dubbed L+FL. Since their weights fell in the range of 99-363 g, the estimated yield of relevant material from female plants only would have been in the range of 0.8-3.3 ton ha^{-1} (dry weight).

Soil and Plant Analysis: Data Processing

Soil

Soil characteristics are presented in Table 1 together with the references for the analytical methods employed.

Plant

A 0.3 g dry material aliquot was mineralized by a conventional nitric-perchloric acid (HNO_3+$HClO_4$) digestion procedure. The elements were determined in soil extracts and plant digests by atomic absorption spectrometry (AAS). The flame technique was employed except for Mo whose low concentration called for the grafite furnace (GF)-AAS.

FIGURE 2. A segment of *Cannabis* stalk. In evidence are a vegetative leaf (L) and an inflorescence (FL). For description of sample preparation see text.

Statistics

For the statistical computations, the SPSS/PC system (1988) was utilized.

RESULTS AND DISCUSSION

To best reproduce the situation encountered with real marijuana samples, no attempt was made to free fresh plant material from adhering particles and the so-called dust elements [iron (Fe), aluminum (Al), silicon (Si), titanium (Ti), and lead (Pb)] were excluded from this study with the exception of Fe. The homogeneity of the plant samples after milling was preliminarily controlled. Two L and two FL fractions were sorted out at random and analyzed for all elements

TABLE 1. Physical and chemical properties of the soils (sampling depth: 0-40 cm).

Soil location	Sand	Silt	Clay	Organic Matter[b]	C.E.C.[c]	$pH_{W,1:2}$	Na[d]	K[d]	Ca[d]	Mg[d]	Cu[e]	Zn[e]	Fe[e]	Mn[e]	Mo[f]
	%			g%g	meq%g		ppm								
Este[a]	14	48	38	2.06	34.1	7.9	33	132	6076	241	8.4	2.02	25	10	0.11
Diamantina	20	54	26	1.53	17.0	8.0	77	104	2889	272	3.3	0.68	18	9	0.21
Ostellato	26	46	28	3.92	26.8	7.7	43	243	4498	356	7.5	2.59	53	12	0.11
Anzola	22	56	22	1.55	18.4	8.0	24	145	3167	138	5.0	1.37	17	14	0.17

[a]Each 0.33 ha portion of this field, where a single *Cannabis* vairety (Carmagnola, Fibrimon, or Uniko) was grown, was sampled separately for soil analysis; since no dishomogeneity was revealed, results were averaged.

[b]Analytical method in Nelson and Sommers, 1982.

[c]Analytical method in Rhoades, 1982.

[d]Exchangeable cations; analytical method in Thomas, 1982.

[e]Extractable cations; analytical method in Gaines and Mitchell, 1979.

[f]Total Mo; analytical method in Hesse, 1971.

TABLE 2. Test of homogeneity for *Cannabis* samples. Two leaf (L) and two flower (FL) samples out of sixty were analyzed (four replicates). Results are given as relative standard deviation (RSD, %).

Sample No.	Sample Type	Replicates No.	Na	K	Ca	Mg	Cu	Zn	Fe	Mn	Mo
			RSD, %								
1	L	4	1.5	3.3	1.0	0.5	2.9	1.3	0.8	2.7	8.1
2	L	4	5.7	1.9	0.9	1.2	3.1	5.1	1.6	1.9	12.6
3	FL	4	4.2	2.0	1.3	1.7	1.9	1.6	1.2	3.7	9.4
4	FL	4	3.0	2.8	2.0	0.7	4.4	3.9	0.5	2.3	7.3

(four replicates). Table 2 gives the level of homogeneity achieved in terms of %RSD (i.e., the analytical variability, V_{anal}) which was considered satisfactory because, with the exclusion of Mo due to the more sensitive but less precise analytical technique adopted for this element, %RSD was generally and often substantially better than 4%. The analytical work was thereafter extended to the remaining samples to give the picture summarized in Table 3. Since none of the fields considered had reportedly ever shown fertility problems with other crops, steadily grown under NPK fertility conservation practices, these data likely depict a general sufficiency status of the plants for the macro as well as micro-nutrients.

Predictably, every population exhibited conspicuous differences between the mean elemental concentrations of L (c_L) and FL (c_{FL}) for most elements and these differences are often larger than those between homologous material of different populations. In particular, when systematic t-tests between c_L and c_{FL} were performed, significant differences (P=0.05) emerged in 67% of all possible comparisons. When this was the case, the elements Ca, Mg, Fe, and Mo preferentially accumulated in the leaves, while the elements Cu, Mn, and Zn levels were more frequently higher in the flowers. Sodium and K were responsible for 55% of the cases where no significant differences were found. This would seem to render the harvesting method for marijuana, i.e., the relative quantities of leaves, flowers, and other plant parts possibly forming the final product, a variability factor capable, if operating within the areas dedicated to this form of production, a factor that would make inorganic composition unreliable in principle as a basis for geographical discrimination. In practice, the relevance of this difficulty which presents itself likewise when sample comparisons are based on cannabinoid content, should be mitigated by the fact that the harvesting method is uniform by tradition within each major growing country (De Faubert Maunder, 1976). Of

course, when considering small size marijuana samples extracted from unmixed lots, as is the practical case, plant part composition will play an important role in establishing the amount of uncertainty attached to the analytical outcome.

In our simulation of marijuana production, the variability of nutrient concentrations among the L as well as the FL fractions of the same crop is the sum of several factors, conceivably: i) the natural variability peculiar to the strain, ii) residual soil heterogeneity and other positional influences, iii) the variability due to the way the various fractions were made up, that is compounding tissues of different ages whose relative proportions likely differ from one fraction to the other, and iv) the analytical variability (V_{anal}), which includes the effects of the uncleanness of the harvested material, its imperfect mixing during the sample preparation, and eventually the instrumental errors. As for the F+FL fractions, they are affected by an additional source of variability, the L to FL weight ratio which is peculiar to each fraction. If the %RSD values calculated from the data in Table 3 were examined, they would appear to be quite low for L and more so for FL and L+FL, being most frequently lower than 9% for all elements; the few exceptions being Na in nearly all cases, Cu in Population 1 (L, 16.8%) and Population 2 (L, 38.4%; FL, 19.9%), Mn in Population 1 (L, 39.3%; FL, 24.0%), and Mo in Populations 1-3. Hence, being V_{anal}'s contribution to total variability in the range 1-4% in terms of RSD for all elements but Mo (Table 2), all other variability sources altogether turn out to add a scant 5-8% to total RSD. As for the noted exceptions, aerial contamination seems a likely explanation, at least for Na and Cu.

When the six populations were compared by ANOVA for element concentrations in L, FL, and L+FL, significant differences were found among populations for every element. By Scheffe's procedure these differences were localized and the results are summarized in Table 4. It can be seen that significant differences (P=0.05 and 0.01) arose for all possible population pairs and they were generated mostly by four to eight elements. The efficiency of any given element as a marker of differences among productions may be expressed by the number of significant contrasts generated by the element as a fraction of all possible contrasts. Such efficiencies (%) can be derived from Table 4 (both levels of significance included):

L: Ca(87), Mo(73), K(67), Fe(60), Mn and Cu(47), Mg and Zn(40), and Na(27)
FL: Mo(73), Ca and Fe(60), Zn and Mn (53), K and Mg(47), Cu(40), and Na(20)
L+FL: Ca(93), Mo(67), K and Fe(60), Zn(53), Mn(47), Na and Cu(40), and Mg(20)

Finally, the possibility of distinguishing the *Cannabis* populations by describing them with respect to all variables (elemental concentrations) simultaneously was probed. The discriminant analysis technique (Lachenbruch, 1975) was applied to the experimental (L, FL) and calculated (L+FL) data. For each population, five canonical discriminant functions were derived in succession, having the form:

$$D_{km} = b_0 + b_1X_{1km} + b_2X_{2km} + \text{--------} + b_pX_{pkm}$$

TABLE 3. Mineral composition of leaves (L), flowers (FL) and leaves+flowers (L+FL) of six *Cannabis* populations grown in Italy. Values (**g%g and *$\mu g\ g^{-1}$ ±standard error) are averages of five samples. For sampling technique, sample preparation and population identification see text.

Population	Na*	K**	Ca**	Mg**	Cu*	Mn*	Fe*	Zn*	Mo*
				Leaves (L)					
1	23±1.8	2.33±0.060	6.6 ±0.20	0.55±0.021	27 ±2.1	50 ±8.8	162±8.4	27.5±0.38	1.9 ±0.20
2	20±2.2	2.06±0.018	7.28±0.091	0.64±0.023	31 ±5.3	36.5±0.71	158±4.7	28 ±1.7	1.8 ±0.10
3	24±1.4	1.82±0.064	5.99±0.093	0.69±0.015	17.5±0.48	31 ±1.3	192±3.7	31 ±1.3	0.74±0.075
4	28±2.1	2.17±0.034	2.8 ±0.10	0.59±0.011	10.2±0.39	22.9±0.95	174±4.4	30.1±0.93	0.90±0.033
5	24±1.5	2.40±0.033	3.85±0.054	0,57±0.009	11.3±0.36	24 ±1.2	194±6.3	41 ±1.8	2.69±0.095
6	36±3.2	2.64±0.058	4.2 ±0.11	0.52±0.015	15.1±0.25	78 ±5.0	274±5.2	34.1±0.83	1.18±0.042
				Flowers (FL)					
1	26 ±1.1	2.32±0.047	2.89±0.078	0.44±0.010	22.4±0.55	61 ±6.5	141 ±4.7	47 ±1.7	1.50±0.097
2	26 ±1.5	2.31±0.086	3.46±0.097	0.46±0.017	26 ±2.3	46 ±1.7	141.5±0.84	43.8±0.81	1.43±0.092
3	25 ±1.8	1.77±0.043	3.09±0.068	0.54±0.010	20.0±0.22	43 ±1.9	144 ±3.7	46 ±1.4	0.93±0.058
4	29.7±0.94	2.19±0.029	2.17±0.093	0.52±0.007	16.2±0.36	34.3±0.85	149 ±2.8	44 ±1.7	0.89±0.043
5	28 ±1.9	2.15±0.036	2.54±0.030	0.50±0.005	16.8±0.23	32 ±2.0	177 ±4.2	59.1±0.93	2.33±0.056
6	34.7±0.72	2.53±0.032	3.04±0.072	0.52±0.009	20.7±0.14	93 ±1.8	254 ±9.2	55.6±0.55	1.35±0.051

Leaves+Flowers (L+FL)

1	24.3±0.64	2.33±0.053	4.9 ±0.13	0.50 ±0.012	25 ±1.1	55 ±7.8	152±5.9	37 ±1.0	1.7 ±0.15
2	23 ±1.3	2.18±0.052	5.4 ±0.10	0.56 ±0.021	29 ±3.8	41 ±1.0	150±2.5	36 ±1.2	1.62±0.08
3	24.7±0.85	1.78±0.053	4.15±0.06	0.594±0.009	19.1±0.35	39 ±1.4	162±3.1	40 ±1.2	0.89±0.042
4	29 ±1.2	2.18±0.031	2.5 ±0.10	0.548±0.009	13.7±0.37	29.6±0.88	160±3.1	38 ±1.6	0.90±0.038
5	26 ±1.4	2.25±0.034	3.07±0.034	0.533±0.006	14.6±0.29	29 ±1.6	184±1.8	52 ±1.2	2.48±0.058
6	35 ±1.1	2.57±0.045	3.48±0.045	0.522±0.009	18.9±0.19	88 ±3.0	256±5.9	47.4±0.60	1.3±0.040

TABLE 4. Elements whose concentrations are significantly different at P=0.01 (underlined) and P=0.05 for the two populations and plant parts indicated.

Population pairs	Soil	Variety	Leaves (L)	Flowers (FL)	Leaves+Flowers (L+FL)
1 - 2	same	different	K, Ca, Mg	Ca	Ca
1 - 3	"	"	K, Mg, Fe, Mo	K, Mg, Mn, Mo	K, Ca Mg, Mo
2 - 3	"	"	K, Ca, Cu, Fe, Mo	K, Mg, Cu, Mo	K, Ca, Cu, Mo
1 - 4	different	same	Ca, Cu, Mn, Mo	Ca, Mg, Cu, Mn, Mo	Ca, Cu, Mn, Mo
1 - 5	"	"	Ca, Cu, Mn, Fe, Zn, Mo	Mg, Cu, Mn, Fe, Zn, Mo	Ca, Cu, Zn, Mn, Mo
4 - 5	"	"	Ca, Zn, Mo	Fe, Zn, Mo	Ca, Fe, Zn, Mo
1 - 6	different		Na, K, Ca, Cu, Mn, Fe, Zn, Mo	Na, Mg, Fe, Zn, Mn	Na, K, Ca, Mn, Zn, Fe, Mo
2 - 6	"		Na, K, Ca, Mg, Cu, Mn, Fe, Mo	Na, Ca, Mg, Cu, Mn, Fe, Zn, Mo	Na, K, Ca, Cu, Mn, Fe, Zn
3 - 6	"		Na, K, Ca, Mg, Mn, Fe	Na, K, Mn, Fe, Zn, Mo	Na, K, Ca, Mg, Mn, Fe, Zn
4 - 6	"		K, Ca, Mn, Fe	K, Ca, Mn, Fe, Zn, Mo	Na, K, Ca, Mn, Fe, Zn
5 - 6	"		Na, Mn, Fe, Zn, Mo	K, Ca, Mn, Fe, Mo	Na, K, Mn, Fe, Mo
2 - 4	"		Ca, Cu, Mo	Ca, Mg, Cu, Mo	Na, Ca, Cu, Mo
2 - 5	"		K, Ca, Cu, Fe, Zn, Mo	Ca, Cu, Fe, Zn, Mo	Ca, Cu, Fe, Zn, Mo
3 - 4	"		K, Ca, Mg	K, Ca	K, Ca
3 - 5	"		K, Mg,, Ca, Zn, Mo	K, Ca, Fe, Zn, Mo	K, Ca, Mg, Fe, Zn, Mo

where: X_{ikm} = the value of element I concentration for sample m in population k; b_i = coefficients computed according to the "best separation among populations" criterion; D_{km} = the value (score) of the discriminant function for sample m in population k. Restricting the discussion to the two most meaningful functions (functions 1-2), i.e., the one having the largest ratio of among populations to within populations sums of squares for the scores and that having the second best ratio, a two-dimensional subspace is defined where the position of each sample is identified by its discriminant scores calculated on the two functions. Graphically the situation for the three fractions considered is shown by the plots in Figure 3 (the asterisk denotes the position of a population centroid, that is the point which has the population's average scores on each of the discriminant functions as coordinates). Figure 3 qualitatively indicates that the discriminant functions 1 and 2, which together amount for 85.2% (L), 76.6% (FL), and 81.9% (L+FL) of the total discriminating power of the system of five equations, succeeded rather well in separating the populations. Their centroids are generally quite far apart and their territories, with the exception of L+FL of Populations 1-2 and 5-6 do not overlap to any extent. Quantitatively the above picture has been substantiated by verifying (the chi-square transformation of the Wilks' lambda statistic was employed) that differences among population means were real and did not reflect sampling variability. The H_0 hypothesis that the means of functions 1 to 5 were equal was tested and the test was repeated for any possible subset of functions remaining after having discarded the function ranking highest in discriminating power. Even after Step 3 of this routine, the null hypothesis was rejected at a significance level ($P<0.001$).

The degree of population separation obtained by a set of discriminant functions may be judged indirectly by calculating, on the basis of the discriminant scores of each sample, the probability of its belonging to one or the other of the populations under study and then assigning the sample to the population for which membership probability is largest (Cooley and Lohnes, 1986). The resulting rate of correct classification, i.e., the number of samples assigned to the very population to which it belongs divided by the total number of samples, is in fact usually taken as an index of the discriminating performance of the functions. In our case, functions 1-2 gave 100% of the samples (30 out of 30) being correctly classified for L and FL, a very good result if compared to a mere 17% rate of correct classification that would be expected if assignments were made by a purely random procedure. For L+FL, the overall distribution pattern of populations' centroids in the space defined by functions 1-2 is less satisfactory (Figure 3) and a worse definition of swarms' territories can be observed in some cases. In fact four samples were misclassified, giving a correct classification rate of 86%. However, when the discriminant function 3 was also considered, the predictive accuracy reached the 100% level. These error rates in sample allocation were seen not to worsen by improving the classification procedure with the use of the 'jacknife' method (Karson, 1982).

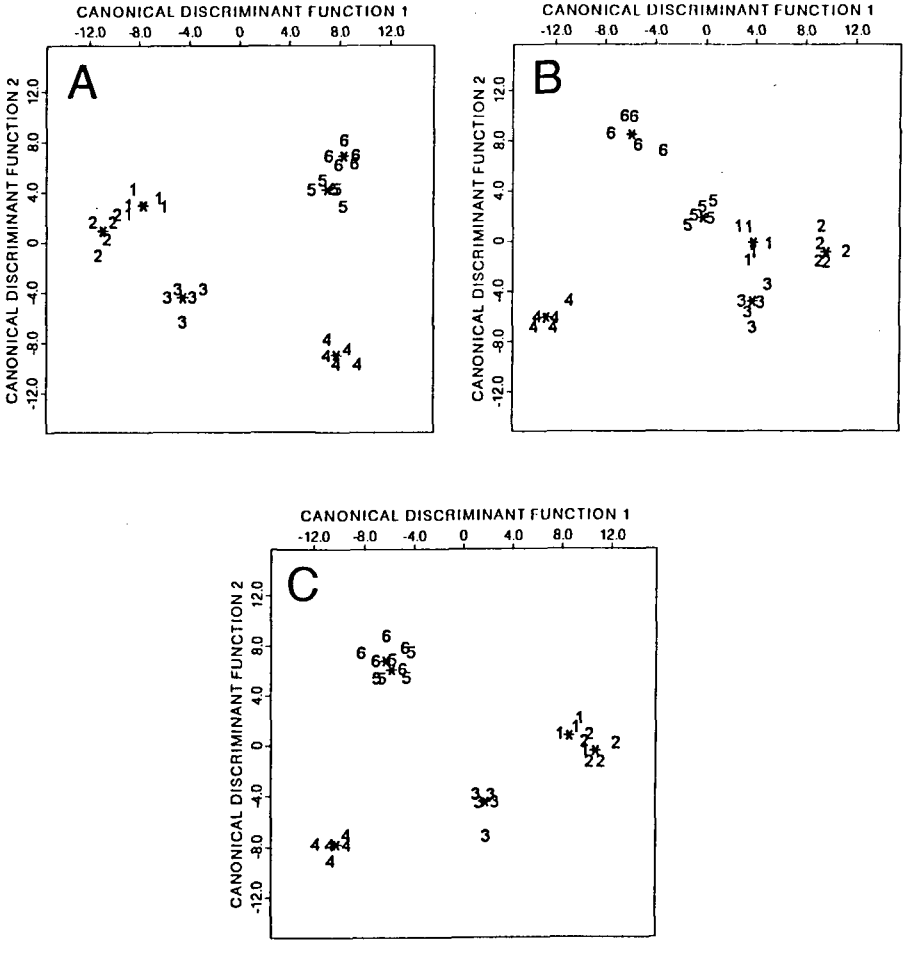

FIGURE 3. Plots of the two discriminant scores for the samples: leaves (L), plot A; flowers (FL), plot B; leaves+flowers (L+FL), plot C; Samples are symbolized by their *Cannabis* population number. When several samples are superimposed, only one symbol is shown. Asterisks indicate the mean scores for each population.

On the basis of these results, the practicability of using suitable discriminant functions in the task of correctly assigning an unknown sample of *Cannabis* material to the very population to which it belongs cannot be ruled out. It does not seem futile, therefore, to explore the degree of consistency of these findings and of all others presented so far. This will be done in a second investigation planned for 1996.

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