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LA THÈSE A ÉTÉ
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FLAVONOIDS OF THE GENUS *MEDICAGO*

by

DEBBIE C. CLASSEN

A thesis
presented to the School of Graduate Studies
of the University of Ottawa
in partial fulfillment of the
requirements for the degree of
Master of Science
in
Biology

Ottawa, Ontario, 1981

DEDICATION

The thesis is dedicated to three very special people who have made it possible for me to complete this work; my dear husband Ted and my parents James and Catherine Fletcher. Their love and understanding were a constant source of encouragement.

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I have been very fortunate to have worked under the guidance of Dr. Constance Nozzolillo during the time it has taken to complete this thesis. I would like to thank her for generously sharing the benefits of her experience. I also greatly appreciate the financial assistance throughout the semesters as well as the allowances provided for conference travel.

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ABSTRACT

Flavonoids from dried leaf tissue of 47 of the approximately 55 species of Medicago were examined using 2-dimensional chromatography on polyamide plates. One species of Melilotus and two species of Trigonella were also studied as these three genera, Medicago, Melilotus and Trigonella are very closely related. Compounds tentatively identified as cyanidin, quercetin, kaempferol, luteolin, apigenin, chrysoeriol, tricin and coumestrol were found. Proanthocyanidins were not detected in the leaf tissue of any of the species of Medicago, Melilotus or Trigonella. Apigenin was found in all species of Medicago except M. cretacea but not in the one species of Melilotus or the two of Trigonella studied. Two compounds designated #23 and #24 occurred only in Medicago. All of the flavonoids were detected as aglycones with the possible exceptions of glucuronides and C-glycosides which are strongly resistant to hydrolysis. Of the phenolics detected, 27 were used as characters in numerical analyses. The results are presented in the form of dendrograms of the average linkage analysis, minimum spanning trees, principal coordinate analyses and as maximal connected subgraphs, all based on the simple matching coefficient. The chemistry reflected the three major groupings of Medicago ;

the annual group Spirocarpos, the perennial group Falcago and the group of unusual species tending towards Trigonella. The flavonoid chemistry of Medicago proved to be of taxonomic importance. The chemical relationships were highly consistant with morphological relationships for Subgenus Medicago, Subgenus Orbicularia and several controversial species. However, results were relatively poor for the Subgenus Spirocarpos. The two species of Subgenus Lupularia, which are morphologically quite similar, proved to be highly divergent chemically. The single species of Melilotus and the two species of Trigonella were dramatically different from Medicago in their flavonoid composition.

RÉSUMÉ

Les flavonoïdes extraits de feuilles desséchées de 47 des 55 espèces de Medicago ont été examinés à l'aide de chromatographie à deux-dimensions sur plaques de polyamide. Une espèce de Melilotus et deux espèces de Trigonella ont aussi été étudié, car ces trois genres: Medicago, Melilotus et Trigonella sont très apparentés. Une série de composés provisoirement identifiés comme cyanidine, quercétine, kaempférol, lutéoline, apigénine, chrysoériol, tricine et coumestrol ont été retrouvés à travers tout le genre. Les proanthocyanidines n'ont pas été detectées dans les tissues des feuilles d'aucune des espèces de Medicago de Melilotus ou de Trigonella. L'apigénine a été découvert dans toutes les espèces à l'exception de M. cretacea mais n'a pas été trouvé dans l'espèce de Melilotus ni dans les deux espèces de Trigonella étudiées. Deux composés appeles #23 et #24 ont été decouvert dans Medicago seulement. Tous les flavonoides ont été détectés comme des aglycones à l'exception des glucuronides et des C-glycosides. L'hydrolyse a été effectué pendant quatre heures mais les glucuronides, qui sont rapportés présent dans le genre Medicago, sont extrêmement résistant à l'hydrolyse et les C-glycosides subissent une isomérisation et non une hydrolyse sous ces conditions. Des

phénoliques détectées, 27 ont été utilisées comme caractères dans les analyses numériques. Les résultats ont été présentés sous forme de dendrogrammes et de graphiques dérivés de tests statistiques modernes. La chimie reflète les trois principaux groupements de Medicago, le groupe des annuelles Spirocarpos, le groupe des vivaces Falcago et le groupe des espèces peu communes se rapprochant de Trigonella. L'analyse numérique révèle une bonne corrélation entre la structuration du groupe Falcago et du groupe des espèces ressemblant à Trigonella basée soit sur la morphologie, soit sur la chimie des flavonoïdes. Quoi qu'il en soit, la chimie des flavonoïdes du groupe Spirocarpos relie les espèces différemment dont le fait la morphologie bien qu'il y avait des affinités parmi les espèces traditionnellement groupées comme Pachyspirae. Quelques-unes des espèces qui ne se groupent pas là où la morphologie les plaçait traditionnellement, ne se regroupent pas nonplus selon d'autres résultats soit de phytoalexine, de pollen, soit d'information serologicale ou florale. Le groupe Lupularia nécessite des études plus approfondies à cause de la divergence des modèles de flavonoïdes et des données de différentes sources.

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Section I

INTRODUCTION

Some aspects of the taxonomy of the genus Medicago are well understood, however, there are major disagreements concerning a number of species. To gain additional information in the hope that it would aid in delimiting the genus more clearly, a chemotaxonomic study involving the genus Medicago and a very small sample from the two closely related genera Trigonella and Melilotus was undertaken.

A study of the chemistry of Medicago would be of interest to both taxonomists and plant breeders. A better description of the species of Medicago would simplify the identification of introduced plants and would provide information on the variation within the genus. This would aid hybridizations for improving crop lines(Bolton 1962). Chemotaxonomic studies may indicate possible crosses that could reduce or eliminate estrogenic compounds and saponins both of which have undesirable affects on livestock.

The genus Medicago is a member of the Leguminosae, a large and economically important family(Harborne 1967).

Medicago species that are naturalized in the Ottawa area are M. sativa, (escaped from cultivation) and M. lupulina (Gleason and Cronquist 1973, Gillett and White 1978).

M. sativa, more commonly known as alfalfa, is said to be the only forage crop cultivated before recorded history, which makes it difficult to determine accurately its origin (Bolton 1962), but it probably originated in Iran (Bolton et al. 1972). Alfalfa was transported to Greece along with the Persian legions who grew it to feed their horses and fatten their cattle. It eventually spread to Rome and then throughout Europe, South America and Mexico (Hanson 1972). During the gold rush in the 1850's, alfalfa was carried from Chile to San Francisco and from there it spread to other areas of North America (Hanson and Davis 1972). It was first introduced into Canada in 1871 in the province of Ontario (Armstrong et al. 1948).

Alfalfa is largely confined to temperate regions and 40% of the total world acreage is grown in North America. In Canada in 1971, total alfalfa seeded, alone and in mixtures was approximately 5.4 million acres with Ontario accounting for close to 1.5 million acres (Anonymous 1971). Seed production is centred in Western Canada.

Most of the alfalfa crop is used as hay and some is used as silage and pasturage, however, the danger of losses from bloat restricts its use as a pasture crop. Nonetheless, alfalfa is a valuable protein source for livestock, with early cut plants (bud stage to 10% bloom) having a protein content of 18% or higher. Under normal conditions, good hay or

grass silage can be the cheapest form of feed energy and protein (Anonymous 1973). Members of the genus Medicago other than M. sativa are also used as forage crops. M. lupulina was once grown as forage in Canada and is still sometimes grown in other countries. Numerous annual species are grown for forage in Australia and cultivars of M. littoralis, M. polymorpha, M. rugosa, M. scutellata, and M. truncatula have been selected. Wild plants used for forage are M. arabica, M. laciniata, M. minima, M. orbicularis and M. praecox. Some of these species are also grown in the southern United States.

Alfalfa, because it is a nitrogen fixing plant, can be used to improve soil conditions and it is an excellent honey crop for bees. It can be used to increase the vitamin content of prepared foods and it may even be included in the human diet. In China, the young leaves are eaten as a vegetable (Hendrick 1919, McCollum et al. 1939) and alfalfa tea and alfalfa flour are also available. The flour has been used to make cakes, crackers and muffins but the greenish colour makes them undesirable. Other suggested recipes include alfalfa cream soup, alfalfa croquettes, alfalfa pudding and alfalfa souffle.

In the genus Medicago, there is fairly good agreement as to the composition of the four Sections comprising the large and distinctive group of annual species, referred to as Sub-

genus Spirocarpos (Heyn 1963, Lesins and Lesins 1979, Small 1981). There is also good agreement that the two species in the Subgenus Lupularia, M. lupulina and M. secundiflora, are closely related morphologically and should be separated from all other species. There is moderately good agreement that that another large group of species, the perennials referred to as Subgenus Medicago deserve separate recognition. There is some disagreement, however, regarding the long-lived perennial M. arborea which is placed in a separate genus by Vassilczenko (1972).

The perennial M. hybrida is considered to be transitional to the genus Trigonella (Small 1981), however, it is closely related to M. suffruticosa, which clearly belongs in Subgenus Medicago. There are several other species whose affinities are more or less intermediate between Trigonella and Medicago. These species include M. cretacea, M. platycarpa, M. pubescens and M. ruthenica. Finally, there are a number of species of very uncertain relationships. Some of these species are occasionally placed in Trigonella, often placed in a separate genus Pocockia (Seringe 1825, Boissier 1849), and frequently placed in Medicago. These species include M. carstiensis, M. heyniana, M. orbicularis and M. radiata.

The accurate identification of crop plants is becoming even more important now that patents can be obtained on cultivars. Observable morphological and physiological charac-

ters are often insufficient in themselves for the easy identification of cultivars. Biochemical data are sometimes helpful in the identification of species and cultivars, and sometimes even the parentage of hybrids (Turner and Alston, 1959). Biochemical information can be obtained at early stages in the plant growth cycle and may reflect variation that is not morphologically or physiologically observable (Monties et al. 1976).

Chemical characters that are of taxonomic value tend to have the following characteristics; 1. chemical complexity and structural variability, 2. physiological stability not being easily modified by the environment, 3. widespread distribution and variation between populations considerably greater than within populations, 4. easy and rapid identification (Harborne 1967), 5. correlation with other characters. For the above reasons, many chemical studies devoted primarily to systematic problems in plants use secondary plant products.

Flavonoids fall into the above class and meet all the requirements for use as taxonomic characters. They show great diversity, with 500-600 flavonoids having been characterised (Harborne 1971). Qualitatively, flavonoids are environmentally and physiologically stable (Alston and Turner 1963, Buttery and Buzzell 1973), however light and the age of plant may cause quantitative differences (Singh and Thompson

1961, Stathem and Crowden 1974). With regards to distribution, flavonoids occur throughout the angiosperms, gymnosperms and pteridophytes (Harborne 1967).

Some biochemical techniques require long and delicate methods which makes their large scale use difficult. However, low molecular weight compounds that can easily be separated by chromatography, such as flavonoids, alkaloids and terpenes, are well suited to chemotaxonomic studies. Other analytical factors that make flavonoids good to work with are that they are chemically stable (Harborne 1975), and relatively simple to extract. Pure compounds can be quantified using the spectrophotometer (Monties et al. 1976). Also, their chromatography can be rapid and consistantly reproduced and a number of colour reactions can be used to characterise them (Smith 1960, Siekel 1964, Dass and Weaver 1972, Niemann 1979).

Flavonoids are C-15 compounds consisting of two benzene rings connected by a three-carbon chain. They have been found in practically every species of higher plant examined (Harborne 1967). The various types of flavoncids are distinguished by the oxidation states of the C-3 chain. The most reduced type of flavonoid examined in this study is the proanthocyanidin; the flavones and isoflavones are more oxidised and the flavonols are the most highly oxidised (Figure 1) (Robinson 1975).

Phenolic compounds in Medicago species have been studied by several authors. Relationships in annual species of Medicago were analysed by Simon (1967) and Simon and Goodall (1968). They reported no clear correlation between the phenolic chemistry and the morphology of the species studied.

Flower colour inheritance in diploid and tetraploid alfalfa was reviewed by Barnes (1966) and free and bound phenolic acids of M. sativa were studied by Newby et al. (1980).

Isoflavonoid phytoalexins of alfalfa have been examined by Dewick and Martin (1976) as well as by Ingham and Millar (1973). Phytoalexins are phenolic substances that higher plants are induced to produce in response to microbial invasion. They are not detectable or are found in low concentrations in healthy plants. Ingham (1979) studied the isoflavonoid phytoalexins of 25 species of Medicago.

The isoflavones daidzein, formononetin, genistein and biochanin A produce estrogen-like responses and along with coumestrol probably contribute to reproductive disturbances in animals whose diet contains a high percentage of alfalfa. These compounds have been studied in forages including M. sativa by Adler and Trainer (1960) and by Guggolz et al. (1961).

Most classes of flavonoids have been reported to occur in alfalfa alone. The flavonols kaempferol and quercetin (Barnes 1966) and the flavones tricin (Ferguson et al. 1950), 4',7-dihydroxyflavone (Bickoff et al. 1965a), 3',4',7-trihydroxyflavone (Bickoff et al. 1965c), chrysoeriol (Harborne 1967) and luteolin (Harborne 1979) are represented. Isoflavones are almost exclusively found in the Leguminosae (Harborne 1971) and those reported to occur in M. sativa include daidzein, formononetin, genistein and biochanin A (Guggolz et al. 1961).

Isoflavonones, pterocarpans and isoflavans are all closely related to isoflavones. Representatives of each, namely, sativone as an isoflavanone (Ingham 1979), medicarpin as a pterocarpan (Smith et al. 1971) and vestitol and sativan as isoflavans (Ingham 1977) all occur in alfalfa.

Cooper and Elliot (1964) report the presence of the anthocyanidins delphinidin, petunidin and malvidin in M. sativa petals. Phenolic acids reported by Newby et al. (1980), include sinapic, ferulic, p-coumaric, vanillic, p-hydroxybenzoic and salicyclic acids. The chalcone isoliquiritigenin and the flavanone liquiritigenin occur in alfalfa as reported by Ingham (1979). M. sativa lacks proanthocyanidins in the leaves according to Bate-Smith (1954) but other members of Medicago are reported to possess them (Gibbs 1974).

Several unusual plant constituents appear to be biogenetically related to the flavonoids and may be classed either with the furanocoumarins or with the flavonoids. Coumestrol is the best-known of this group known as coumestans (Robinson 1975). Coumestrol (Bickoff et al. 1969), medicagol (Livingston et al. 1965), lucernol and sativol (Spencer et al. 1966a) are all coumestans and are reported to occur in alfalfa. Other coumestans reported are the 3,9- dihydroxy-8-methoxy (Spencer et al. 1966b) and the 3-hydroxy-9-methoxy-coumestans (Bickoff et al. 1965b). This brief literature survey indicates that over 30 phenolic compounds have been reported as occurring in the species M. sativa alone. The present study undertaken is primarily concerned with flavonols, flavones, isoflavones, anthocyanidins and proanthocyanidins.

Within a particular taxonomic group, the presence of certain unusual compounds may help delimit the group. Sometimes chemical features can be used to speculate about evolutionary affinities but many secondary compounds are too widely distributed among groups which have no evolutionary ties for this to be possible. For example, carbon-glycosides of flavones may reflect one to several independent evolutionary lines (Alston et al. 1963). Similar parallelisms also occur in the cytology, morphology and physiology of plant groups.

The groupings of Medicago according to Lesins and Lesins(1979) are found in Table 1 with species of controversial positions marked with an asterisk. This classification based on traditional characters will be used for comparison with the chemical results because it is the most recently published classification to include both perennial and annual groups as well as the Trigonella -like species. [The Heyn(1963) classification involves only the annual Spirocarpos group]. Small (1981) follows this classification fairly closely except that he does not acknowledge the Subgenus Orbicularia but instead places M. cretacea, M. platycarpa and M. heyniana in their own monotypic groups. Small also segregates M. arborea into its own group and places M. noeana in with the Pachyspirae rather than in the Rotatae group.

Numerical taxonomic techniques were used to evaluate the chemical data collected. Numerical taxonomy is defined by Sneath and Sokal (1973) as the grouping by numerical methods of taxonomic units into taxa on the basis of their character states.

Numerical taxonomy has several advantages, 1. it allows subtle relationships to be objectively evaluated, 2. it allows the assessment of variation in a large number of taxa to obtain patterns of correlation(Briggs and Walters 1969), 3. it can integrate data from a variety of sources such as morphology, physiology and chemistry 4. it is more effi-

cient since a large amount of the taxonomic process can be automated(Sokal and Sneath 1966) 5. it forces workers to use more and better described characters.

The taxa used in numerical taxonomic studies, be they cultivars, species, genera etc. are referred to as operational taxonomic units(O.T.U.'s). In this study the O.T.U. is the species.

All of the numerical techniques used in this study required that "similarity coefficients" be calculated between the O.T.U.'s. Similarity coefficients are a quantification of the resemblances between the O.T.U.'s(Sneath and Sokal 1973). There are a great number of similarity coefficients that could be used. In this study the Jaccard and simple matching coefficients were calculated. The data in chemotaxonomic studies is most often recorded as presence of a compound (and represented by the number 1) or as absence(and represented as 0). The information recorded in this was is used to calculate the similarity coefficients as shown in Figure 2. The difference between the Jaccard and the simple matching coefficient is that the former ignores co-occurring absences while the latter takes them into account(Sneath and Sokal 1973).

Several basic numerical taxonomic techniques were employed to evaluate the chemical data collected. These included ordination by principal coordinate analysis(Sneath

and Sokal 1973) and SAHN (sequential, agglomerative, hierarchic, non- overlapping) clustering methods (Sneath and Sokal 1973). The graphical methods of the minimum spanning tree and maximal connected subgraphs were also used. Finally, a technique for comparing the data from studies of different sets of characters of exactly the same set of O.T.U.'s (Gower 1971) was used to compare the flavonoid data collected in this study with morphological data for the same species reported in the literature (Small 1981, Small et al 1981a,b). Methods in this area of numerical taxonomy are not yet well developed.

Ordination analysis refers to a set of multivariate techniques applied to O.T.U.'s scored for numerous characters in such a way that the variation is simplified. Simplification is based on finding a reduced number of axes of variation. It often happens that the first two or three axes provide a simplified and useful picture of the variation pattern which was incomprehensible in its original form. The technique adopted here, known as principal coordinate analysis, was developed by Gower (1966). It is good for assessing large group trends but poor for assessing closely related O.T.U.'s. Cluster analysis has exactly the reverse properties. Therefore the two techniques compensate for each others differences.

The most widespread set of techniques used in numerical taxonomy are the SAHN techniques referred to above. Average linkage clustering falls into this category of numerical methods. As with most SAHN techniques in current use the first step consists of grouping the two O.T.U.'s which exhibit the smallest dissimilarity, then grouping of the next least dissimilar pair is carried out. When linkage of a new O.T.U. is proposed then its distance to each member of the previously formed cluster is considered. The O.T.U. fuses with the cluster or simple O.T.U. giving the shortest mean distance. This process is continued until all O.T.U.'s are joined into a group (Clifford and Stephenson 1975). The resulting branched figure is known as a dendrogram and illustrates in hierarchic fashion the relationships of the O.T.U.'s. It is known that different clustering methods, as well as different similarity coefficients may produce different results. Therefore it is important that additional techniques be used to confirm the findings.

The minimum spanning tree is a very useful graphic representation of relationships. A graph is a set of points whose relationships are expressed in terms of lines connecting the points. Once again the two O.T.U.'s with the smallest dissimilarity are linked and then succeedingly larger dissimilarities are used as a basis for further linking. However, no closed loops are allowed to form. The procedure continues until all O.T.U.'s are linked. The resulting

graph represents the shortest direct path joining all the O.T.U.'s. The minimum spanning tree reveals which O.T.U.'s are most similar to other O.T.U.'s and may act as a check for any distortion in the principal coordinate analysis when superimposed on the latter.

Maximal connected subgraphs are graphs linking O.T.U.'s at or below a given level of dissimilarity. At selected relatively low levels of dissimilarity the figure can very usefully portray relationships but at high levels of dissimilarity so many linkages may be present that the figure is difficult to interpret.

Flavonoid variation in 47 species of Medicago, one species of Melilotus and two species of Trigonella, was analysed using these numerical taxonomic techniques.

TABLE 1

The Taxa of Medicago

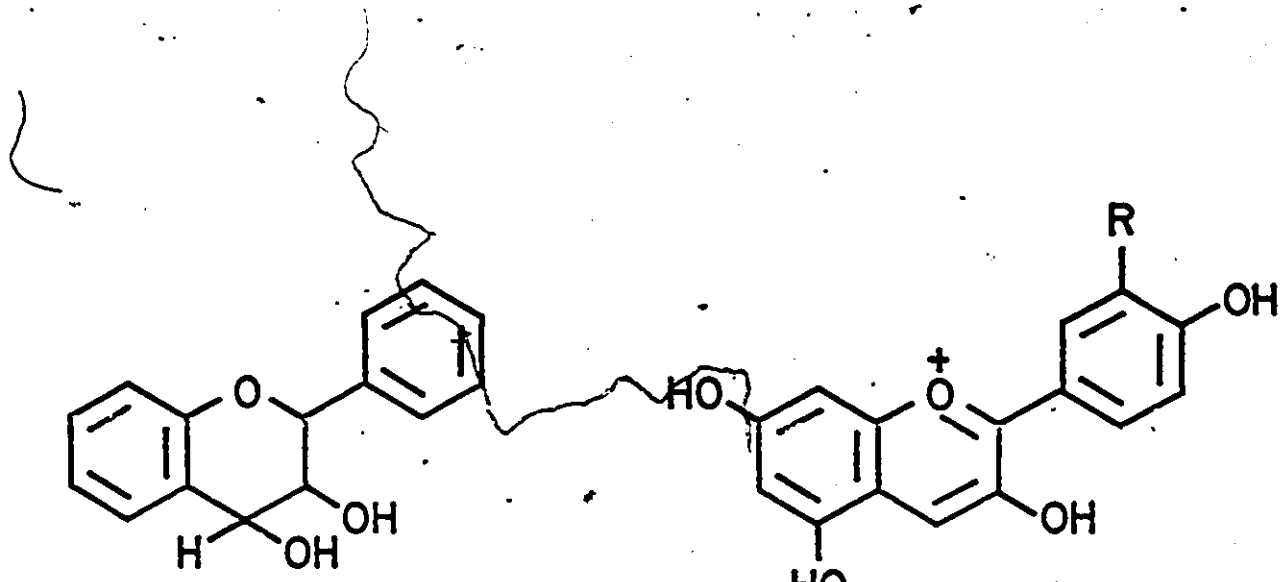
Subgenus Medicago	Section Arboreae	M. arborea*
	Section Falcago	M. cancellata
		M. daghestanica*
		M. pironae*
		M. prostrata
		M. sativa
		M. saxatilis
	Section Marinae	M. marina
	Section Suffruticosae	M. hybrida
		M. suffruticosa
Subgenus Lupularia		M. lupulina
		M. secundiflora
Subgenus Orbicularia	Section Carstiensae	M. carstiensis*
	Section Cretaceae	M. cretacea*
	Section Heynianae	M. heyniana*
	Section Hymencarpos	M. radiata*
	Section Orbicubares	M. orbicularis
	Section Platycarpae	M. platycarpa*
		M. ruthenica*
		M. pubescens*
Subgenus Spirocarpos	Section Leptospirae	M. arabica*
		M. coronata
		M. disciformis
		M. laciniata
		M. lanigera
		M. minima
		M. polymorpha*
		M. praecox*
		M. sauvagei
		M. tenoreana
	Section Fotatae	M. blancheana
		M. noeana*
		M. rotata
		M. rugosa*
		M. scutellata*
	Section Intertextae	M. granadensis
		M. intertexta
		M. muricoleptis
	Section Pachyspirae	M. constricta
		M. doliata
		M. littoralis
		M. murex
		M. rigidula
		M. soleirolii*
		M. tornata
		M. truncatula
		M. turbinata

*=species whose position is in dispute

Groupings are those of Lesins and Lesins 1979 except for M. pubescens which was not treated in that work. It should be noted that Small(1981) places M. noeana in Section Pachyspirae and not in Section Fotatae.

The major classes of flavonoid aglycones studied were the proanthocyanidins, the anthocyanidins, the flavones, the isoflavones and the flavonols.

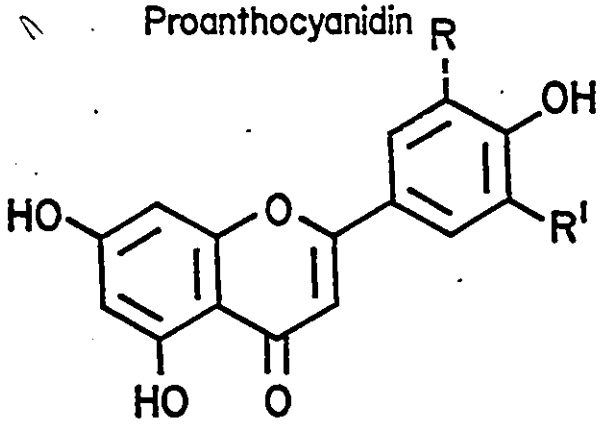
Figure 1: Classes of Flavonoids.



Proanthocyanidin

Anthocyanidin

R=OH ; cyanidin



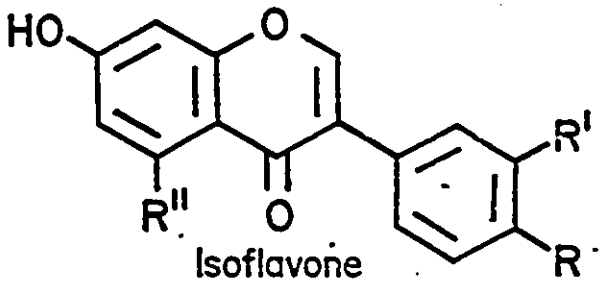
Flavone

R=R'=H ; apigenin

R=OH, R'=H ; luteolin

$R=OCH_3$, R'=H ; chrysoeriol

R=R'=OCH_3 ; tricin



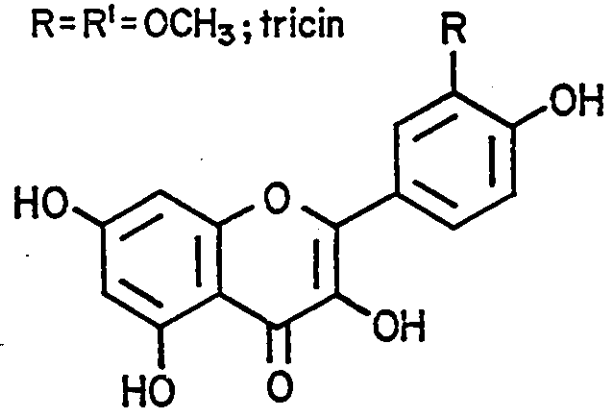
Isoflavone

R=OH, R'=R''=H ; daidzein

$R=OCH_3$, R'=R''=H ; formononetin

R=R''=OH, R'=H ; genistein

$R=OCH_3$, R'=H, R''=OH ; biochanin A



Flavonol

R = H ; kaempferol

R = OH ; quercetin

The Jaccard coefficient does not use scores of (0,0) when calculating similarity coefficients but the simple matching coefficient does.

Figure 2: Similarity Coefficients used in the Numerical Taxonomic Analysis.

Jaccard coefficient

$$\frac{a}{a + b + c}$$

Simple matching coefficient

$$\frac{a + d}{a + b + c + d}$$

a = joint score of (1,1)

b = joint score of (0,1)

c = joint score of (1,0)

d = joint score of (0,0)

Section II

MATERIALS AND METHODS

2.1 PLANT MATERIAL

2.1.1 Species Study

Of the approximately 55 species of Medicago, 47 were available for sampling as well as one species of Melilotus and two of Trigonella. All of the Medicago species were grown from seed (Appendix Table 8). Most of the species were grown in six inch peat pots outdoors at the Central Experimental Farm, Ottawa, during the summer of 1979. A number of species, however, had to be grown in the greenhouse (minimum 11 hour photoperiod) in order for flowering to occur (Figure 3). M. carstiensis is known to require exposure to cold to induce flowering so it was grown in the field during the summer of 1980 and the rhizomes were collected in the fall. These were cold treated for three weeks at 5°C, re-planted and placed in a growth chamber. However, it still failed to flower. Flowering material of M. tenoreana and the two species of Trigonella, T. caerulea and T. corniculata was also unavailable. Voucher specimens of species studied were collected and deposited in the vascular plant herbarium of the Department of Agriculture, Ottawa. [The collections of

vouchers were made by B. Brookes, B.P.I., Agriculture Canada, Ottawa.] The sample of Melilotus alba was from a uniform, wild population near Ottawa and a voucher was also taken.

The samples of each species consisted of leaves of healthy plants at a flowering and fruiting stage (stage 4.5 Figure 4), except for those previously mentioned species which failed to flower and had to be collected at stage 3.9 (Figure 4). The plants were cut off just above ground level and the flowers and fruits were removed. The vegetative material was placed in paper bags in a dryer at 27-28°C for approximately 48 hours. After this time the samples were removed, the leaves were stripped from the stem and the latter ground to a powder. The samples were stored in "whirl pack" bags in the dark.

2.1.2 Intraspecies Study

An attempt was made to collect at least two populations of each species. However, this was only possible with 26 of the 47 Medicago species.

2.1.3 Developmental Study

Additional leaf material was collected from three species, M. arborea, M. lupulina and M. sativa for use in a developmental study. Cotyledons and the first

leaf(unifoliate) from the seedlings(stage 2.0 Figure 4) of each of the M. lupulina and M. sativa species were removed and dried separately. A vegetative plant with approximately nine branches(stage 3.9 Figure 4) was collected from the M. arborea and M. sativa species. Plants at stage 4.5(Figure 4) were sampled from all three species. The plants were dried and the leaves were stripped, ground and stored as above.

2.2 EXTRACTION METHODS

Each of the ether, ethyl acetate and anthocyanidin extracts were prepared using the same 0.5±.01 g of powdered leaf tissue(Figure 5). The powder was mixed with 3.0 ml of 2N HCl and 3.0 ml of 95% ethanol in a 15 ml screw top test tube and placed in a 100°C water bath for 250 minutes (Harborne 1965). At the end of this time all O-glycosides should have been completely hydrolysed(Harborne 1965). This flavonoid extract was allowed to cool, was centrifuged and the supernatant was decanted. The residue from some species was retained for an alternate test for proanthocyanidins.

2.2.1 Ether Extract

Approximately 6.0 ml of distilled water was added to the flavonoid extract supernatant to render the chlorophyll insoluble. The chlorophyll was removed by centrifugation and

discarded. The flavonoid extract was extracted twice with 0.5 ml of diethyl ether.

2.2.2 Ethyl Acetate Extract

The flavonoid extract was then extracted with 1.0 ml of ethyl acetate. The ether and ethyl acetate extracts were examined for flavonoids using thin-layer chromatography.

2.2.3 Isoamyl Alcohol Extract

The final extraction of the flavonoid extract was with 1.0 ml isoamyl alcohol. This extract would contain any anthocyanidins present which were identified using paper chromatography.

2.2.4 Water Extract

A water extract of each population was prepared using 1.0 g of dried leaf material immersed in 10 ml of distilled water. The water solution was centrifuged and here the supernatant was decanted and filtered through glass wool to remove fine particles that did not sediment during low speed centrifugation. The supernatant was extracted with 1.0 ml of isoamyl alcohol to remove any anthocyanidins present. The solution remaining was used to test for proanthocyanidins using column chromatography to separate them from other compounds and boiling in acid for their detection.

2.3 ANALYTICAL METHODS

2.3.1 Alternate Method for Testing for Proanthocyanidins

Four populations did not have enough leaf material available to test for proanthocyanidins using the water extracts so an alternate method was used to test them.

Approximately 3.0 ml of 2N HCl was added to the residue from the initial centrifugation of the flavonoid extract and the colour of the solution was observed. The tube was placed in a 100°C water bath for 15-20 minutes. The tube was removed and approximately 1.0 ml of water was added to the solution and then it was centrifuged. The supernatant was decanted and extracted once with 1.0 ml of isoamyl alcohol to remove any anthocyanidins present presumably due to the hydrolysis of proanthocyanidins. However, with this method, a positive indication (i.e. a red solution) could also be due to anthocyanin aglycones not removed before boiling in 2N HCl.

2.3.2 Thin-layer Chromatography

The ether and ethyl acetate extracts were chromatographed on 75mm X 75mm sheets of polyamide microlayer ML 1515 (Mandel Scientific Co. Ltd.). The volume spotted was approximately 0.01 ml of ethyl acetate extract and approximately 0.02 ml of ether extract. Standards were also spotted for each direction with apigenin, chrysoeriol, luteolin, orien-

tin and vitexin run in one lane, biochanin A, genistein, kaempferol, myricetin, quercetin and tricin run in the middle lane and coumestrol, dihydroquercetin and isorhamnetin run in the outside lane.

The chromatograms were run in chloroform-methanol- butanone (CMB 12:2:1 30 ml) (Egger and Keil 1965) as the first direction, dried and run in the second direction using methanol-acetic acid-water (MAW 18:1:1 40 ml) (Mabry et al. 1970) (Figure 6). The polyamide sheets were dried, examined under ultraviolet light and then sprayed with the flavone reagent, diphenylboric acid ethanolamine complex (1% in water with 0.5 ml of 33% ammonia hydroxide) (Collins 1979) and re-examined under ultraviolet light.

A "limits of detection" test using the standard genistein was performed to examine the sensitivity of this method. The initial solution was made to a concentration of 2×10^{-2} g/ml in methanol and diluted so that concentrations of 1×10^{-2} g/ml, 5×10^{-3} g/ml, 2.5×10^{-3} g/ml, 1.25×10^{-3} g/ml, 6.3×10^{-4} g/ml, 3.1×10^{-4} g/ml, 1.6×10^{-4} g/ml, 7.8×10^{-5} g/ml, 3.9×10^{-5} g/ml, 2.0×10^{-5} g/ml and 1.0×10^{-5} g/ml were obtained. Equal amounts (2.5×10^{-3} ml) of all the above dilutions were spotted on polyamide , run ascendingly by chloroform-methanol butanone (CMB 12:2:1) and then sprayed with the flavone reagent.

2.3.3 Paper Chromatography

The anthocyanidin extracts and the isoamyl alcohol extracts from the alternate test for proanthocyanidins were spotted on Whatmann paper #1 using syringes and a Unimetrics multi-spotter. Standards of cyanidin, malvidin and delphinidin were also spotted and the papers were run descendingly by Forestal(glacial acetic acid-water-hydrochloric acid, 30:10:3 volume/volume).

2.3.4 Column Chromatography

Polyvinylpyrrolidone(PVP) columns were prepared in disposible Pasteur pipettes. The PVP powder was washed with tap water to remove the fine particles. After 10 minutes of settling, the water was decanted and the process was repeated 19 times(Vierma de Bezada 1980). Glass wool was placed in the bottom of the pipettes and PVP suspended in water was pipetted into the column until the bed height was approximately 3 cm. The water extract(5.0 ml) was allowed to run into the column which was then eluted with methanol-1% HCl. This acidic elution mixture makes any anthocyanins visible so their progress through the column could be followed(Figure 7). After the anthocyanins had been eluted from the column, the whole column was blown out from the narrow end using air pressure and collected in a paper towel(Thakur 1980).

The top 1 cm of the column was cut off, transferred to a test tube and boiled in 3.0 ml of 2N HCl for 10 minutes (Bate-Smith 1954). If proanthocyanidins were present a red colour would appear in the solution due to the formation of anthocyanidins. It should be noted, however, that in some cases anthocyanin aglycones were detected in the water extracts of some species showing particularly high concentrations of anthocyanins. The aglycones appeared as a pink band at the top of the PVP column and could not be eluted with the methanol- 1% HCl. Therefore, when the top of the column was boiled in acid and the solution was initially pink, a colour change to a deeper pink or red colour would have indicated the presence of proanthocyanidins.

2.3.5 Tentative Identification as Flavonoids

Ethyl acetate extracts of a number of species (M. rotata, M. rugosa, M. ruthenica, M. sativa and M. suffruticosa) were spotted on polyamide microlayers. (This combination of species contained the 26 compounds selected to construct the reference pattern.) The chromatograms were run in two dimensions using CMB and MAW as in the other chromatograms but instead of spraying with the flavone reagent, these chromatograms were sprayed with a solution of 1% $FeCl_3$ - 1% $K_3Fe(CN)_6$ in water (Krishnamurty 1980). This spray is also known as Turnball's blue reagent (Dass and Weaver 1972). The compounds were allowed to react for 5 minutes and then the

chromatograms were rinsed under a gentle stream of distilled water until no more colour was washed off. Most phenolic compounds stain dark blue on a white background. However catechol derivatives have a greenish colour (Robinson 1975) (Figure 8). An indication of a more specifically flavonoid nature is obtained by examining the compounds under ultraviolet light before and after spraying with the flavone reagent. This reagent reacts with flavonoids to cause a colour change.

2.4 ANALYSIS OF DATA

2.4.1 Recording of Data

In total, the presence or absence of 28 compounds of each population was recorded. Two of these chemical characters are represented by anthocyanidins (character #27) and by proanthocyanidins (character #28). The remaining 26 compounds were obtained using the ether and ethyl acetate chromatograms and were used to construct a reference pattern (Figure 9) (Table 2). Each chromatogram was compared to the reference pattern and the population was then scored for the presence or absence of each chemical character. Tentative identification of compounds was based on comparisons between the colours of the spots and the colours of the standards in ultraviolet light alone and in ultraviolet light after spraying with the flavone reagent. Rf's of the spots had to be within 10% of the Rf's of the standard compound (Table 3).

2.4.2 Numerical Analysis

The presence of a compound was recorded as a 1 and the absence of a compound was recorded as a 0. The data were processed in the computing facilities of the Engineering and Statistical Research Institute (ESRI) and the Data Processing Division, Finance and Administration Branch, Agriculture Canada using programs in the library of the ESRI.

Jaccard and simple matching coefficients were calculated and used in principal coordinate analysis, average linkage, minimum spanning tree and maximal connected subgraph analyses. Three sets of analyses were conducted. The first analysis used all 50 species studied including the three non-Medicago species. The second analysis involved only members of the Subgenus Spirocarpos (since they tend to be well separated morphologically) and the third used all the remaining species. In the second and third analyses, the three non- Medicago species were not included.

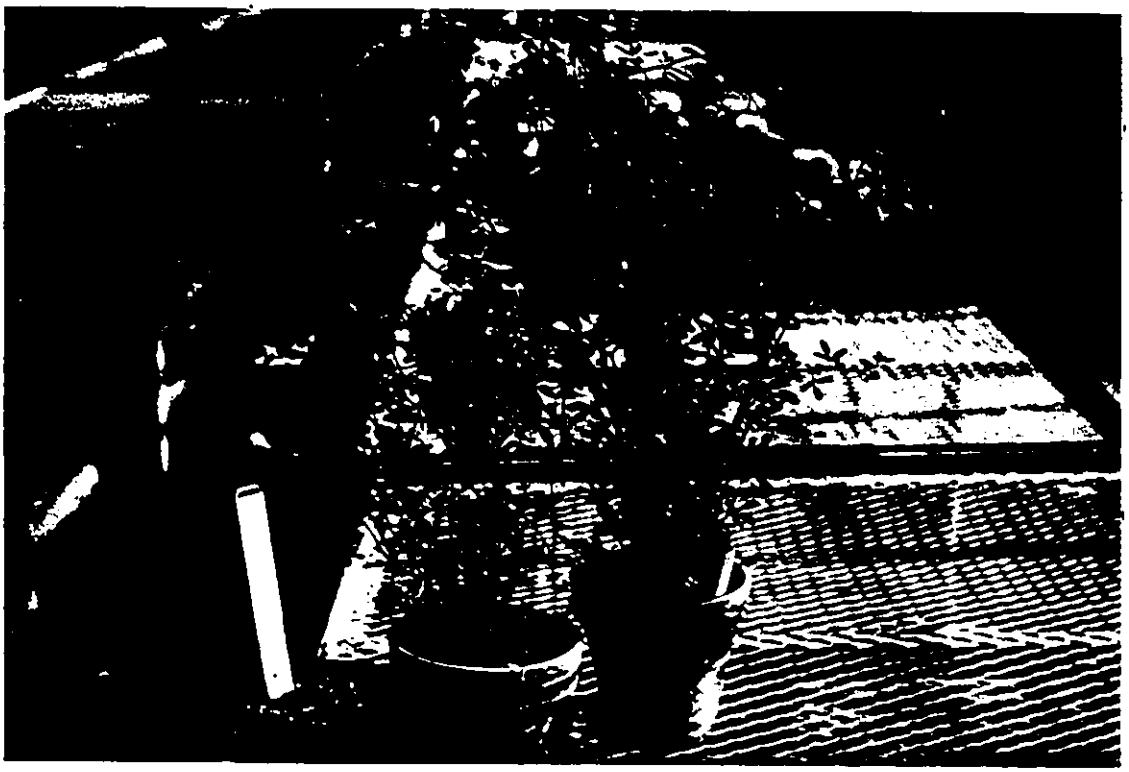
Some species were grown with longer photoperiods in the greenhouse in Ottawa so that flowering and fruiting material could be sampled. The tall, woody branches in the left foreground belong to M. arborea, the only shrub in the genus Medicago.

Figure 3: Medicago Species Under Greenhouse Conditions at the Central Experimental Farm, Ottawa.

COLOURED PICTURES

The seedling plant consisted of the two cotyledons and the first leaf(which was unifoliate) and was referred to as stage 2.0. The middle pot contained a strictly vegetative plant, consisting of approximately nine main branches and all trifoliate leaves. This represented stage 3.9. The pot on the far right contained a mature plant of all trifoliate leaves, bearing both flowers and fruit. This represented stage 4.5 which was the stage collected for most species for flavonoid analysis.

Figure 4: The Developmental Stages of M. sativa.



COLOURED PICTURES

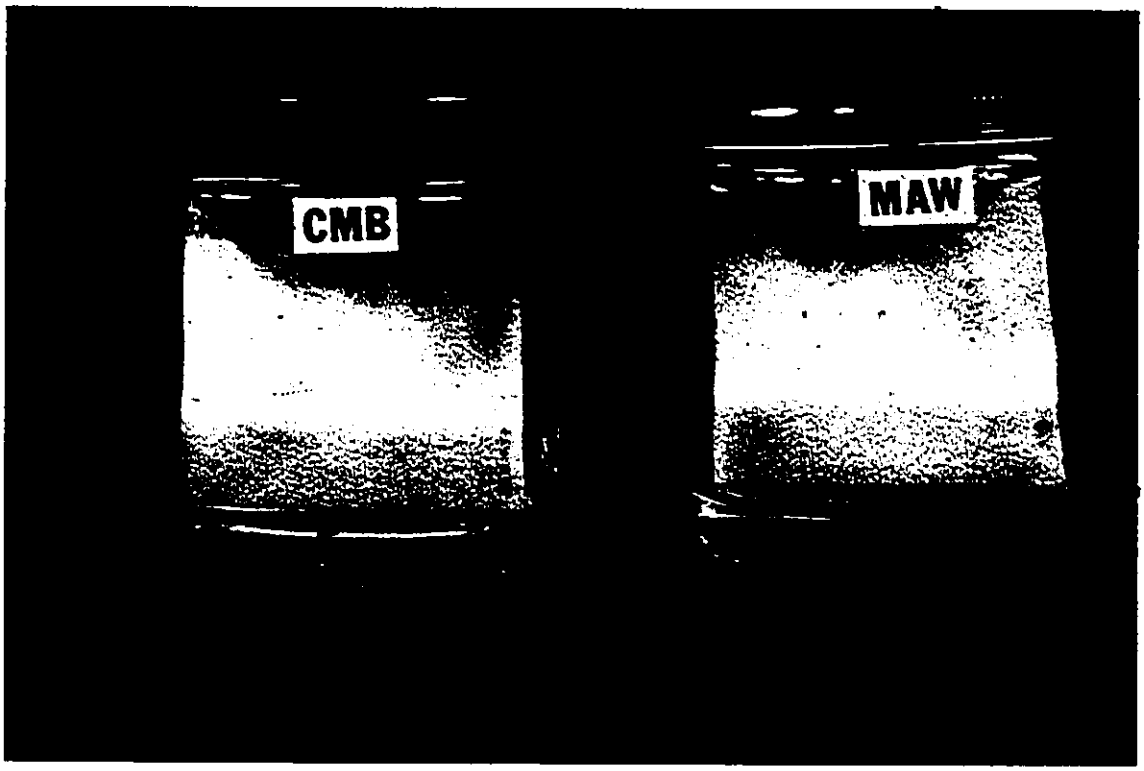
After hydrolysis of the leaf tissue for 250 minutes in 2N HCl and 95% ethanol, ether, ethyl acetate and isoamyl alcohol extracts were obtained for chromatographic analysis.

Figure 5: Initial Leaf Tissue and Subsequent Extractions Obtained for Analysis.

COLOURED PICTURES

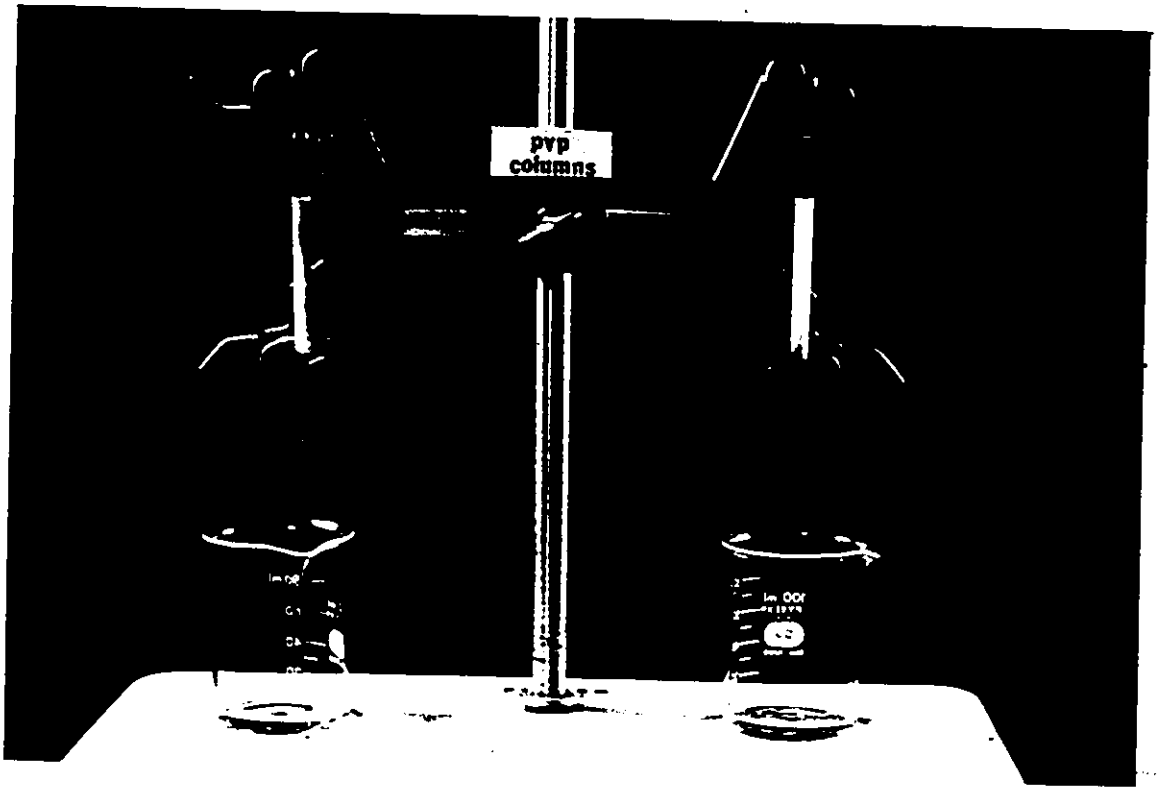
Four hundred ml jars were used as chambers for the chromatography of 75mm X 75mm polyamide plates. The first direction consisted of 30 ml of chloroform-methanol-butanone (CMB 12:2:1) and the second direction consisted of 40 ml of methanol-acetic acid-water (MAW 18:1:1).

Figure 6: Polyamide Microlayer Chromatography.


CMB
MAW

Water extracts from dried leaf material were placed on PVP columns and eluted with methanol-1% HCl. The acidic elution solvent allowed the visualization of any anthocyanins(the pink at the top of the column). When the anthocyanins had been eluted, the top of the column was boiled in 2N HCl to determine if proanthocyanidins were present.

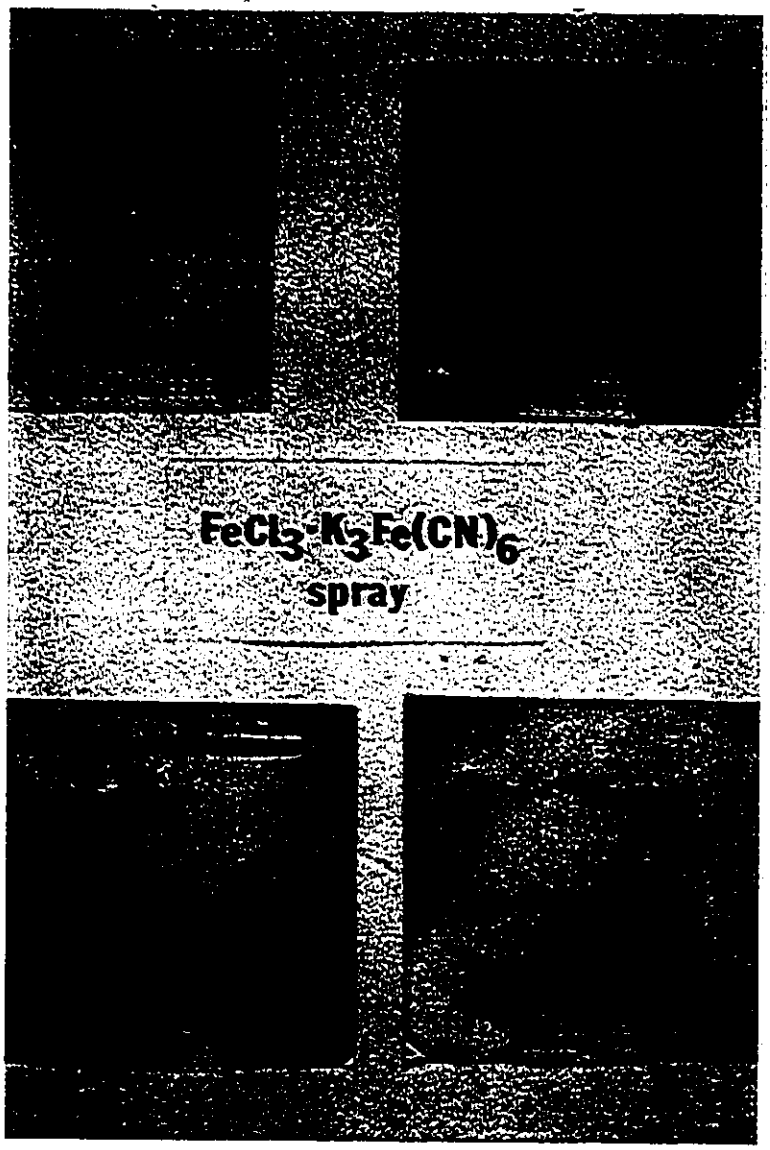
Figure 7: Polyvinylpyrrolidone(PVP) Columns.



COLOURED PICTURES

Most phenolic compounds appear as blue spots after being sprayed with $FeCl_3-K_3Fe(CN)_6$.

Figure 8: Chromatograms Sprayed with 1%$FeCl_3-K_3Fe(CN)_6$.



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COPIE DE QUALITEE INFERIEURE

COLOURED PICTURES

Twenty-six compounds [all but 1(tentatively identified as coumestrol) were phenolics] were used to construct a basic pattern to which each chromatogram was compared.

Figure 9: Reference Pattern of Compounds Studied.

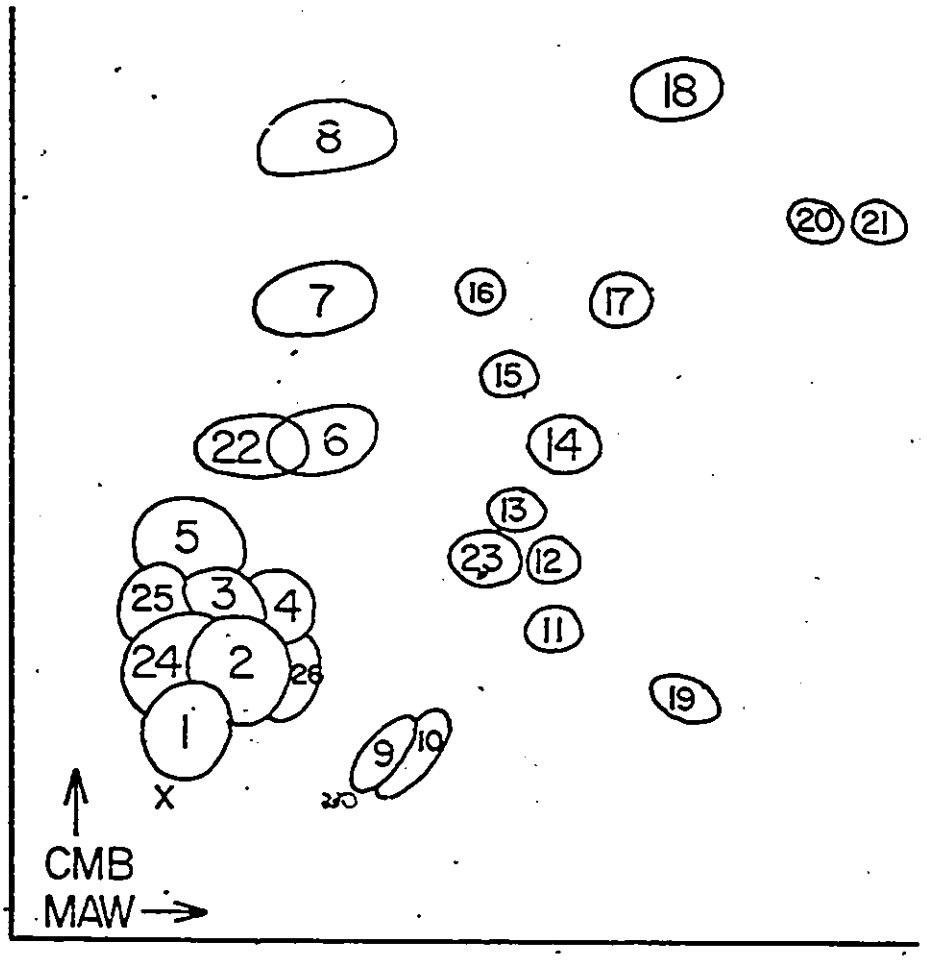

18
8
20
21
7
16
17
15
22
6
14
13
5
23
12
25
3
4
11
24
2
26
19
1
9
10
X
CMB
MAW

TABLE 2

Characteristics of Reference Spots

Spot Number	Colours		
	UV light only	UV light and flavone spray	$FeCl_3$-$K_3Fe(CN)_6$ spray
1	dull orange	bright orange	blue
2	black	bright yellow	blue
3	orange	green	blue
4	black	dull green	blue
5	invisible	yellow green	blue
6	black	green-brown*	gold
7	black	green-brown*	blue
8	black	gold*	blue
9	dull orange	bright orange	blue
10	black	yellow green	blue
11	dull orange	red orange	blue
12	invisible	pink	blue
13	black	yellow	blue
14	yellow orange	bright green	blue
15	black	dull green	blue
16	black	dull green	blue
17	yellow	bright green	blue
18	black	dull orange	blue
19	invisible	blue	blue
20	invisible	blue	blue
21	invisible	blue	blue
22	yellow	green	blue
23	blue	bright purple	blue
24	black	yellow green	blue
25	bright purple	bright purple	bright purple
26	blue	blue	blue

27 cyanidin
28 proanthocyanidin

*=spots are initially yellow green

TABLE 3

Characteristics of Standard Compounds

Compound	Colours		Rf's		
	UV light only	UV light and flavone spray	CMB	MAW	Spot Number
apigenin [1]	black	green-brown*	.40±.04	.29±.04	#6
chrysoeriol [2]	black	green-brown*	.54±.04	.28±.05	#7
luteolin [2]	black	bright yellow	.19±.02	.22±.03	#2
tricin [1]	black	gold*	.73±.05	.25±.03	#8
kaempferol [3]	yellow orange	green	.20±.04	.18±.03	#3
myricetin [5]	orange	red orange	.04±.01	.13±.03	
quercetin [6]	dull orange	bright orange	.09±.02	.16±.03	#1
biochanin A [4]	black	dull green	.86±.05	.44±.07	
genistein [3]	black	dull green	.43±.04	.36±.05	
orientin [2]	dull orange	bright yellow	.08±.02	.43±.06	
vitexin [2]	black	green brown	.12±.02	.51±.06	
dihydro quercetin [7]	black	orange	.07±.02	.10±.03	
coumestrol [1]	purple	purple	.21±.05	.10±.01	#25

*=spots are initially yellow green

Sources
1. Dr. H.G. Krishnamurty, University of Delhi, India
2. W. Nichols, University of British Columbia
3. K and K Labs
4. Aldrich
5. Fluka
6. Dr. Morita, Central Experimental Farm, Ottawa
7. Koch Labs

Section III

RESULTS

Typical chromatograms of each Section of the genus Medicago are shown in Figure 10. The chromatograms were run in the two solvents as described in the Materials and Methods, sprayed with the flavone reagent and exposed to ultraviolet light.

When sprayed with the $FeCl_3$-$K_3Fe(CN)_6$ solution (Figure 8), all compounds stained the blue colour characteristic of phenolics except for compounds #6 and #25. Compound #6 developed a gold colour while compound #25 retained its bright purple colour. Based on Rf values and colour reactions, these two compounds were tentatively identified as apigenin (#6) and coumestrol (#25). Apigenin belongs to the flavone class of the flavonoids and it has a different staining property than other flavonoids (Dass and Weaver 1972). Coumestrol belongs to the coumestan class of compounds. These compounds are occasionally grouped with the flavonoids because they are biosynthatically related (Robinson 1975). For the above reasons, even though these two compounds did not stain as typical phenolics, they were included in this study. All the compounds (with the exception of coumestrol which is not a flavonoid and compound #26) showed colour changes with the flavone reagent.

As mentioned above, compound #6 was tentatively identified as apigenin and #25 as coumestrol. Compound 1 was tentatively identified as quercetin, #2 as luteolin, #3 as kaempferol, #7 as chrysoeriol and #8 as tricin. Compounds #4, #5, #9-24 and #26 remain to be identified. Characters #27 and #28 represented cyanidin and proanthocyanidins respectively.

Flavonols and flavones both seemed to be common in the genus Medicago. Quercetin and kaempferol occurred in over half the species examined (although not necessarily together) but myricetin was not detected in the genus. The four flavones tentatively identified as apigenin, chrysoeriol, tricin and luteolin proved to be widespread in Medicago. The coumestan, coumestrol was also quite common. The concentrations of the compounds varied noticeably but this aspect of the flavonoid chemistry was merely observed and no attempt was made to quantify this variation.

Biochanin A and genistein (isoflavones), orientin and vitexin (C-glycosides) and dihydroquercetin (flavanonol) could not be detected in any of the chromatograms using the standards available.

The standard of genistein was used in the limits of detection test. On the polyamide microlayers, using the flavone reagent spray, the lowest concentration detected was 2×10^{-5} g/ml. Since only 2.5×10^{-3} ml was spotted, then the

spot detected represented 5 X 10^{-8} g or 0.5 ng of genistein. The same approximate level of detection was assumed for other flavonoids.

The 1.0 ml extract of ethyl acetate represented the flavonoids in 0.5 g of leaf material but only 0.01 ml of the extract was spotted on the chromatogram, therefore each chromatogram actually represented 0.005g of tissue. Given that approximately 0.5 ng of a flavonoid could be detected, if a compound was recorded as present, it occurred in the leaf tissue in a concentration of at least 10 p.p.m.(dry weight).

The variation between populations of the same species was calculated by determining the number of times the two populations disagreed as to the presence of a compound and dividing by the total number of compounds(28). This value was then converted to a percentage(Table 4). The average variation between populations was approximately 14%, however some species revealed much more variation than others. For example, M. intertexta showed 46% variation and M. cancellata, M. carstiensis, M. hybrida, M. prostrata and M. scutellata all showed over 20% variation between populations. Three compounds, #19, #20 and #21 were difficult to detect on chromatograms and could account for up to 11% of the variation. Some of the variation could also probably be attributed to concentration differences. Specimen misidentifica-

tion is still a possible, though remote, source of variation. When assessing a species, if the compound occurred in at least one population, then the species as a whole was scored as possessing the compound. The important consideration was that the species showed the ability to make the compound in at least some portion of its variation pattern.

The chromatograms from the developmental study revealed that even at the seedling stage, the plant possessed a considerable array of phenolic compounds(Figure 11). The Medicago lupulina seedlings had seven flavonoids and one other phenolic while the M. sativa seedlings had eight flavonoids and one other phenolic. In the mature stage(4.5) M. lupulina leaves contained 15 compounds and M. sativa leaves at the same stage had 18. The seedling pattern was found to be entirely contained in the mature leaf pattern with the mature leaf adding compounds to this early pattern. No compounds were observed to disappear with maturity.

The vegetative plant of M. sativa (stage 3.9) and the flowering and fruiting plant(stage 4.5) differed only in the presence of one extra compound in the more mature plant(the flavonoid #16). The other species examined at these two growth stages, M. arborea, showed both stages to have identical flavonoid patterns consisting of 15 compounds. These results were important since a few of the species, as indi-

cated earlier in the Materials and Methods, had to be collected at stage 3.9 while the majority of the species were harvested at stage 4.5.

Anthocyanidins were found to occur in 33 of the 47 Medicago species examined. These 33 species were scattered among all the Sections of the genus. However, no anthocyanidins were detected in the one species of Melilotus (Melilotus alba) or in the two species of Trigonella (T. caerula and T. corniculata) surveyed.

The species that possessed anthocyanidins appeared to have the same aglycone. The aglycone co-chromatographed with a cyanidin standard both on paper in Forestal and on polyamide in MAW.

Proanthocyanidins were not detected in the leaves of any of the Medicago, Melilotus or Trigonella species examined.

The chemical data was compiled in the form of Table 5 and presented to ESRI for numerical analysis. (The complete results using all populations is given in Appendix Table 9).

The calculations performed using either the Jaccard or simple matching coefficient gave virtually identical results. Both the presence and the absence of compounds contributed information regarding relationships so the simple matching coefficient was presented in the results.

Since principal coordinate analysis is good for assessing large group trends, it proved to be best to examine Figure 12 when studying the genus as a whole. The analysis revealed that for the first two axes, the Subgenus Medicago Sections Falcago, Arboreae and Marinae along with Subgenus Spirocarpos Section Pachyspirae tended to separate from the other species of Medicago. The Subgenus Orbicularia grouped at the opposite pole to the Subgenus Medicago group. The Subgenus Spirocarpos Section Leptospirae, Section Rotatae and Section Intertextae along with Subgenus Medicago Section Suffruticosae and Subgenus Lupularia were scattered through the centre.

By connecting the species in the same manner as they were in the minimum spanning tree, relationships were seen more clearly and the distortion that occurred was visualized. From the way the species plotted along the first and second coordinates, it appeared that M. cretacea was the intermediate between the Trigonella and Melilotus genera and the genus Medicago, but with the minimum spanning tree added, it was seen that M. cretacea was the closest Medicago to Melilotus but that it was M. muricoleptis that was the closest Medicago to Trigonella.

In the study of the non- Spirocarpos species alone, the plot of the first two axes of the principal coordinate analysis revealed two broad groups separated along the first

axis (Figure 13). One group was composed of members of the Subgenus Medicago and the other consisted of species of the Subgenus Orbicularia with M. hybrida acting as a link between the two. Here again the over- lay with the minimum spanning tree helps visualize distortion. For example, in the Subgenus Medicago, M. daghestanica and M. marina plotted close together but in fact they are linked as follows; M. daghestanica-M. prostrata-M. sativa-M. arborea-M. marina and not directly as was suggested by their close proximity on the graph.

The plot of the first two axes of the Subgenus Spirocarpos species only (Figure 14) revealed a tendency for the Section Pachyspirae to separate from the remaining species along the first axis. Medicago praecox grouped with the Section Pachyspirae and although M. lanigera also appeared to do so, this was a distortion as it was closest to M. coronata in its relationships according to the minimum spanning tree rather than to M. doliata or M. rigidula as it was plotted.

The results of the average linkage clustering analysis are presented as dendrograms. The dendrogram resulting from the analysis of the non- Spirocarpos species (Figure 15) also revealed good separation of the Subgenus Medicago and the Subgenus Orbicularia as did the principal coordinate analysis. All members of the Subgenus Orbicularia grouped to-

gether except for M. cretacea which is considered a divergent species. M. pubescens which is often considered a Trigonella, fit in with the Subgenus Orbicularia. Medicago suffruticosa and M. hybrida acted as links between the two Subgenera. Traditionally, M. secundiflora and M. lupulina have been placed together as the Subgenus Lupularia but the average linkage analysis had M. lupulina grouped with the Subgenus Orbicularia but M. secundiflora grouped with Subgenus Medicago.

The subgenus Medicago was very cohesive although subgroups were observed. Medicago marina separated off from the remainder of the Subgenus Medicago as did M. arborea while M. pironae, M. prostrata, M. sativa, M. cancellata, M. daghestanica and M. saxatilis clustered together. This latter group of six species all belong to Section Falcago.

The results of the average linkage analysis on the Subgenus Spirocarpos (Figure 16) revealed no clear separations between the four Sections. However, it did suggest some affinities for some controversial species. Medicago noeana and M. soleirolii appeared to be closest in their relationships to members of the Section Pachyspirae.

The minimum spanning tree of the non- Spirocarpos species (Figure 17) once again emphasized the strong species relationships within the Subgenus Medicago and Subgenus Orbicularia. It also confirmed that the Subgenus Lupularia is

composed of two species which are chemically highly divergent.

The minimum spanning tree of the Subgenus Spirocarpos (Figure 18) revealed something that was not evident from the average linkage dendrogram. All the species traditionally placed in Section Pachyspirae except for M. murex and M. constricta showed close relationships. Species of Section Leptospirae formed subgroups of one species (M. tenoreana) or two species (M. laciniata and M. sauvagei, M. lanigera and M. coronata, M. polymorpha and M. disciformis) all joined to M. rotata. In an indirect manner this suggested some structure within Section Leptospirae through M. rotata. Medicago praecox, M. arabica and M. minima are usually considered as members of Section Leptospirae but they did not appear to group well with the others. The species of Sections Rotatae and Intertextae were scattered and only M. rugosa and M. blancheana both of Section Rotatae showed some form of the traditional affinities.

The maximal connected subgraph of the non-Spirocarpos species (Figure 19) again emphasized the many relationships among the Trigonella-like species and among the species of Subgenus Medicago but the few relationships between the two groups.

The same kind of graph for the Subgenus Spirocarpos (Figure 20) revealed a complex set of interrelationships with no clear structure.

An analysis of the amount of agreement between classifications based on the flavonoid data of this study and various subsets of morphological data are given in Table 6. The pollen morphology produced the poorest agreement with any of the other sets of characters. The remaining subsets of morphological characters (vegetative characters, fruit characters and flower characters) were somewhat more consistant with each other than with flavonoid chemistry, but flavonoid chemistry showed much better agreement than did pollen morphology.

Table 7 presents the individual compounds in the species of Medicago, the one species of Melilotus and the two species of Trigonella. Compound #6, tentatively identified as apigenin appeared in all Medicago species except for M. cretacea and did not appear in the Melilotus and Trigonella species. Compounds #23 and #24 also occurred in all Medicago species (including M. cretacea) but these two compounds were absent from Melilotus and Trigonella.

Medicago cretacea was significantly different from other members of Subgenus Orbicularia in that compounds #4, #6, #13, #15 and #27 could not be detected. All members of Subgenus Orbicularia contained compounds #1 (tentatively identified as quercetin), #10 and #11.

Within the Subgenus Medicago the two species in Section Suffruticosae differed from the remaining species in that

they possessed compound #2 (tentatively identified as luteolin), lacked compound #8 (tentatively identified as tricin) and lacked compound #18. Compound #18 was detected only in the Subgenus Medicago and in some members of Subgenus Spirocarpos Section Pachyspirae. All members of Subgenus Medicago contained compound #7 (tentatively identified as chrysoeriol).

TABLE 4

Percent of Intraspecific Variation

M. arabica	4	M. orbicularis	4
M. arborea	11	M. pironae	4
M. blancheana	14	M. platycarpa	14
M. cancellata	21	M. polymorpha	7
M. carstiensis	25	M. prostrata	25
M. coronata	18	M. rigidula	14
M. doliata	11	M. rotata	7
M. hybrida	29	M. rugosa	4
M. intertexta	46	M. scutellata	25
M. littoralis	4	M. suffruticosa	14
M. lupulina	7	M. tornata	18
M. marina	14	M. truncatula	7
M. minima	14	M. turbinata	7

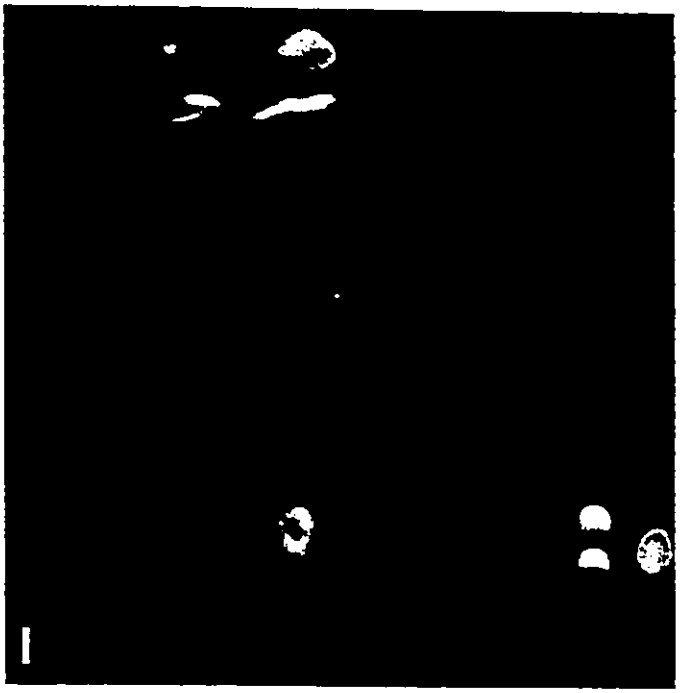
The table gives the per cent variation between the two populations of each of the above species calculated as follows: $\frac{b+c}{a+b+c+d} \times 100$

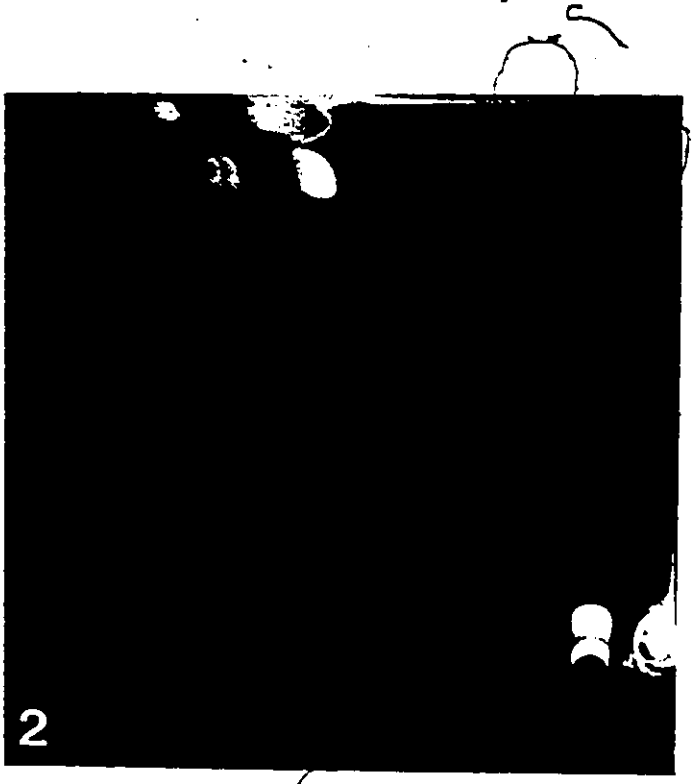
where
a=joint score of (1,1)
b=joint score of (1,0)
c=joint score of (0,1)
d=joint score of (0,0)

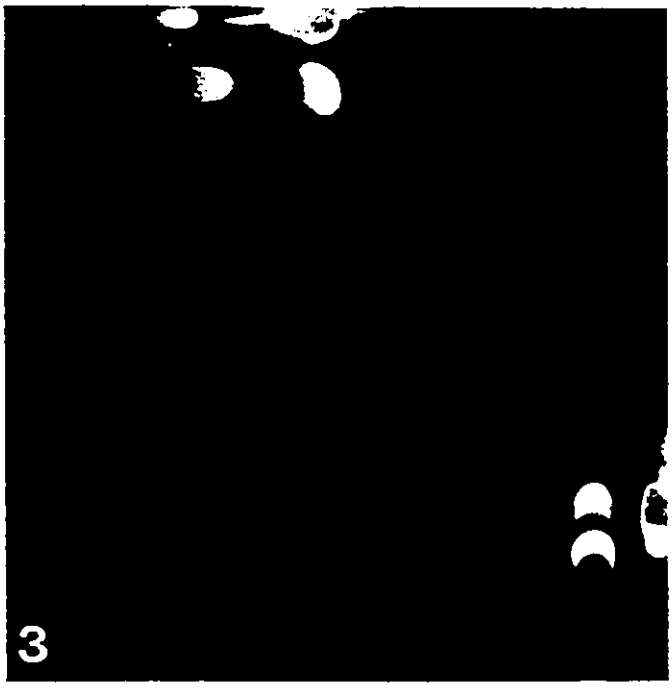
1 Subgenus Medicago Section Arboreae
2 Subgenus Medicago Section Falcago
3 Subgenus Medicago Section Marinae
4 Subgenus Medicago Section Suffruticosae
5 Subgenus Orbicularia Section Heynianae
6 Subgenus Orbicularia Section Cretaceae
7 Subgenus Orbicularia Section Carstiensae
8 Subgenus Orbicularia Section Hymenocarpos
9 Subgenus Orbicularia Section Orbiculares
10 Subgenus Orbicularia Section Platycarpae
11 Subgenus Orbicularia
12 Subgenus Spirocarpos Section Leptospirae
13 Subgenus Spirocarpos Section Rotatae
14 Subgenus Spirocarpos Section Intertextae
15 Subgenus Spirocarpos Section Pachyspirae
16 Subgenus Lupularia
17 Subgenus Lupularia
18 Genus Melilotus
19 Genus Trigonella

The extracts were chromatographed on polyamide, sprayed with the flavone reagent and illuminated by ultraviolet light.

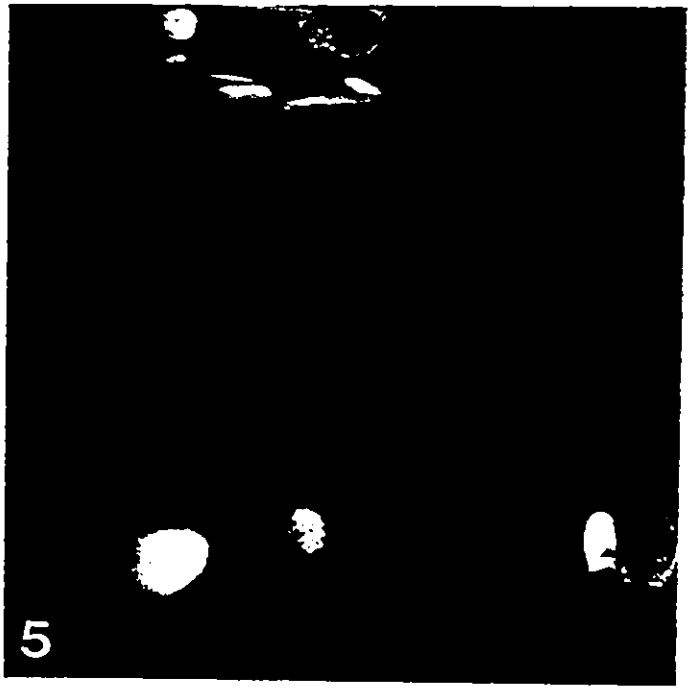
Figure 10: Typical Chromatograms Representing the Genus Trigonella, Genus Melilotus and Each Section of the Genus Medicago.

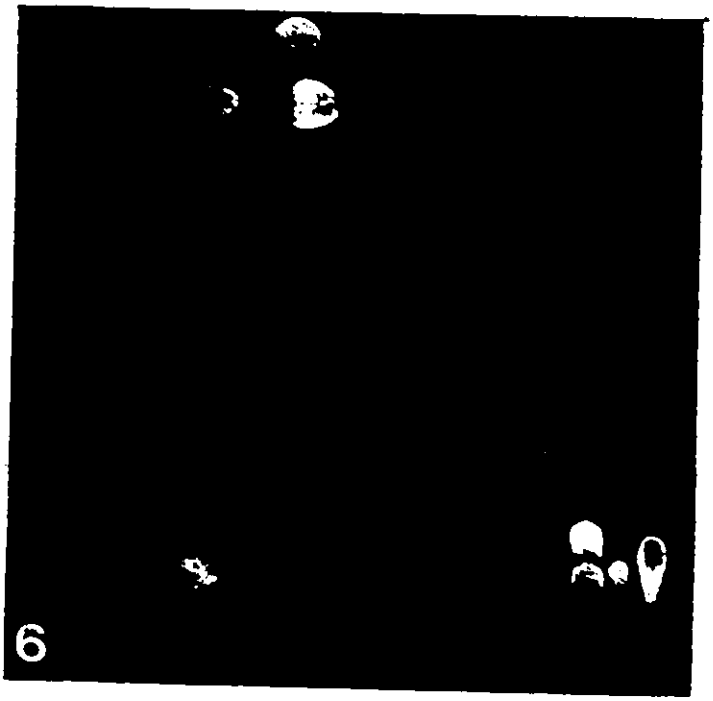


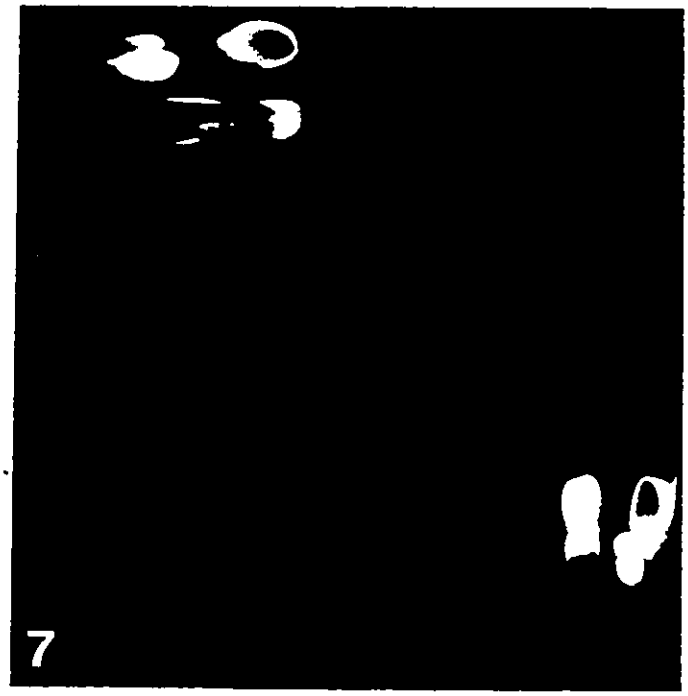


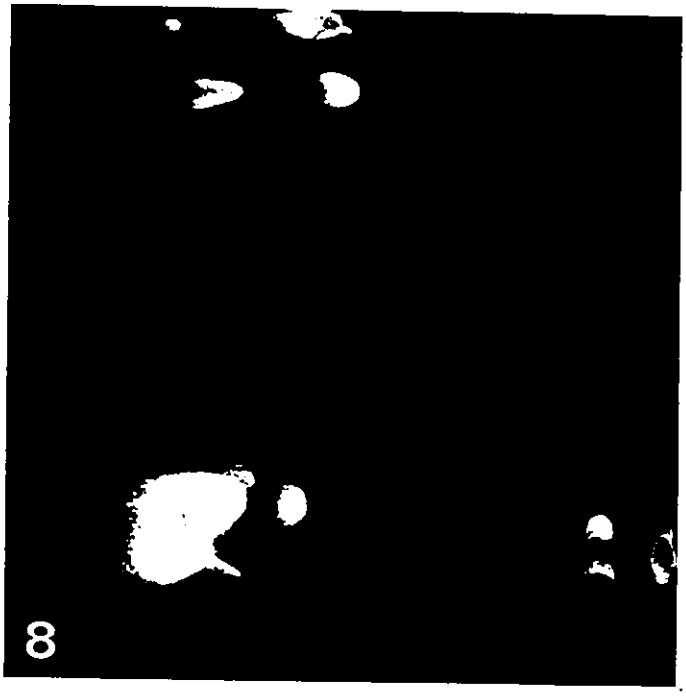


COLOURED PICTURES

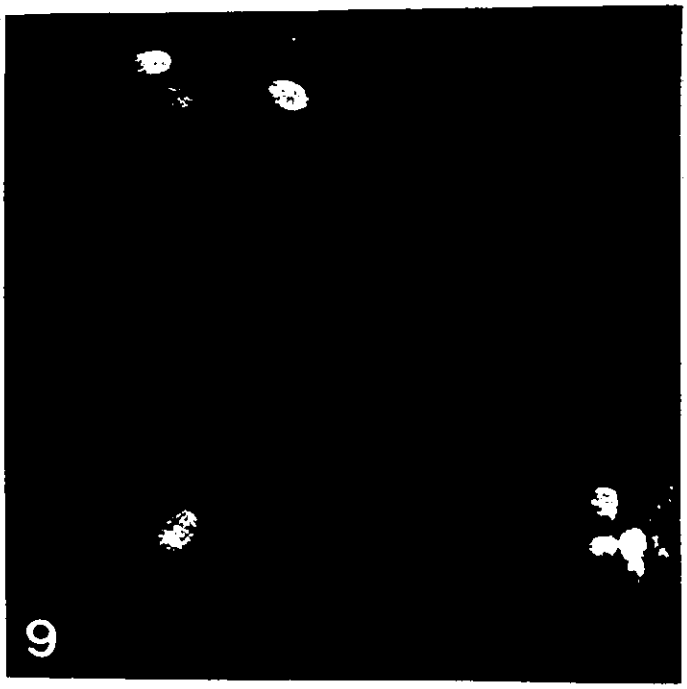


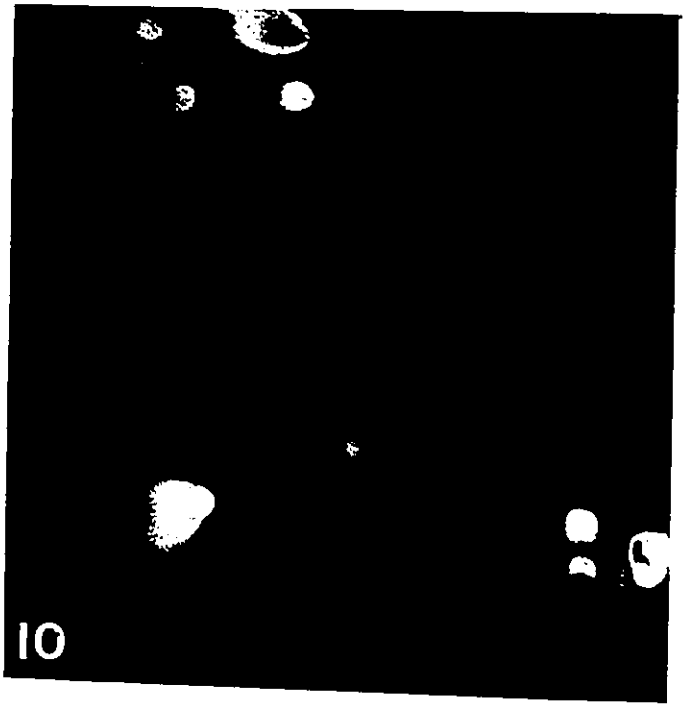


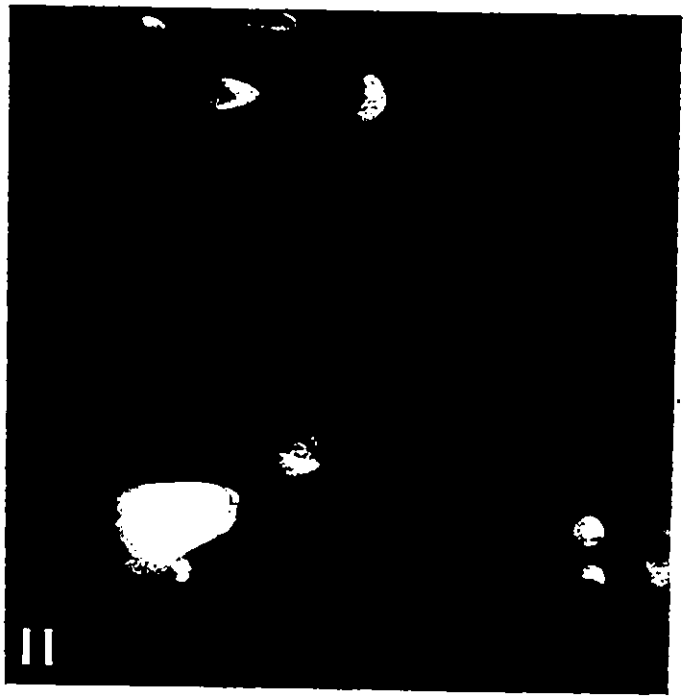




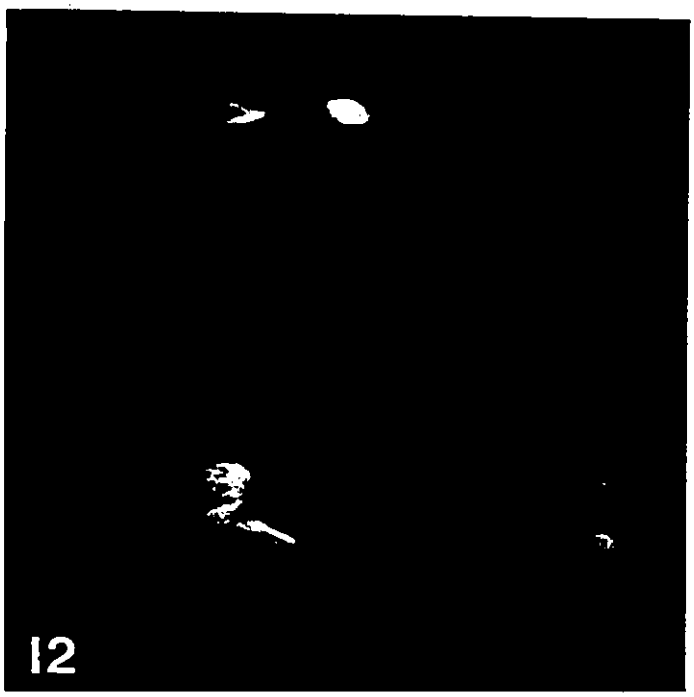
COLOURED PICTURES

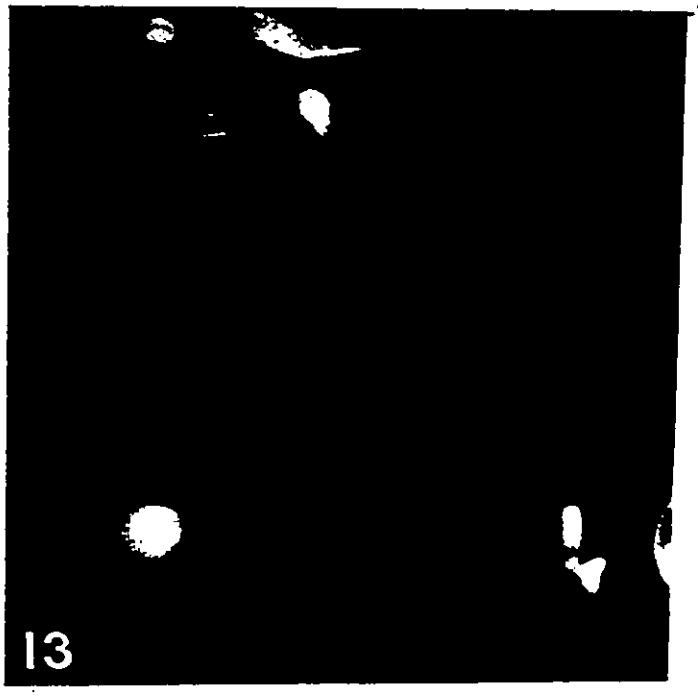


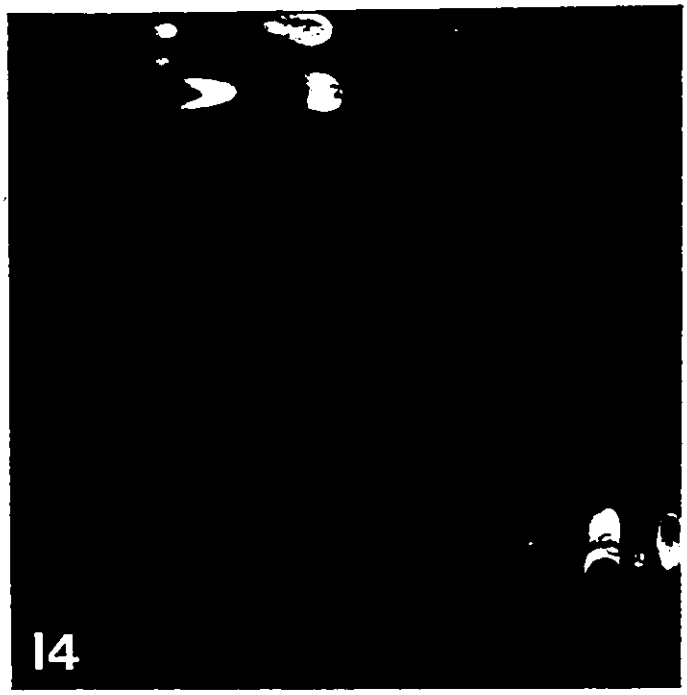


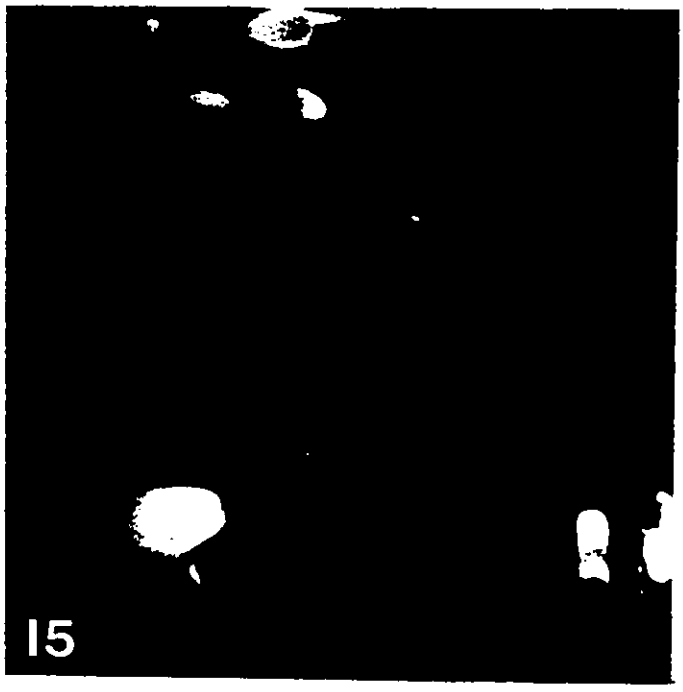


COLOURED PICTURES

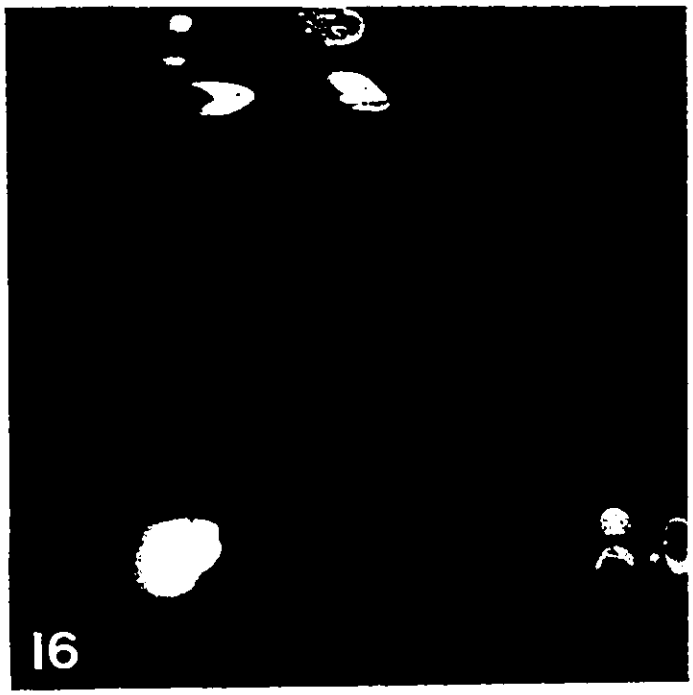


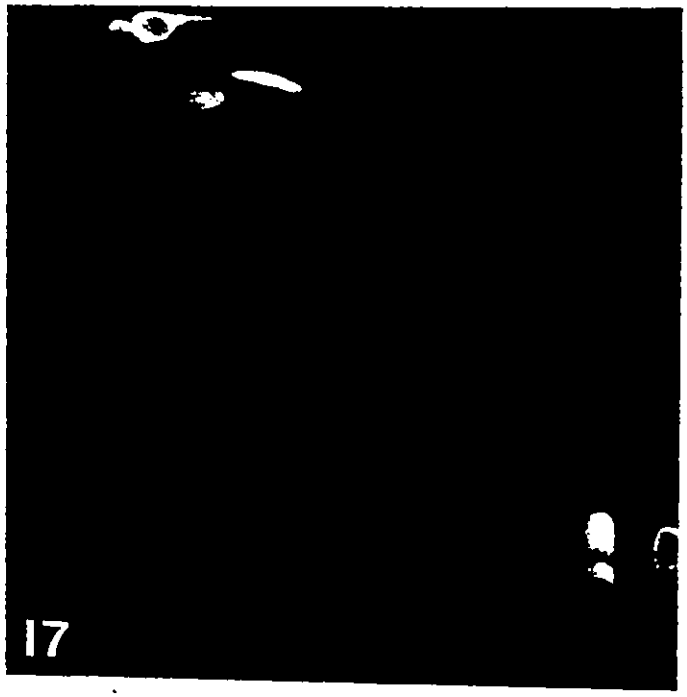


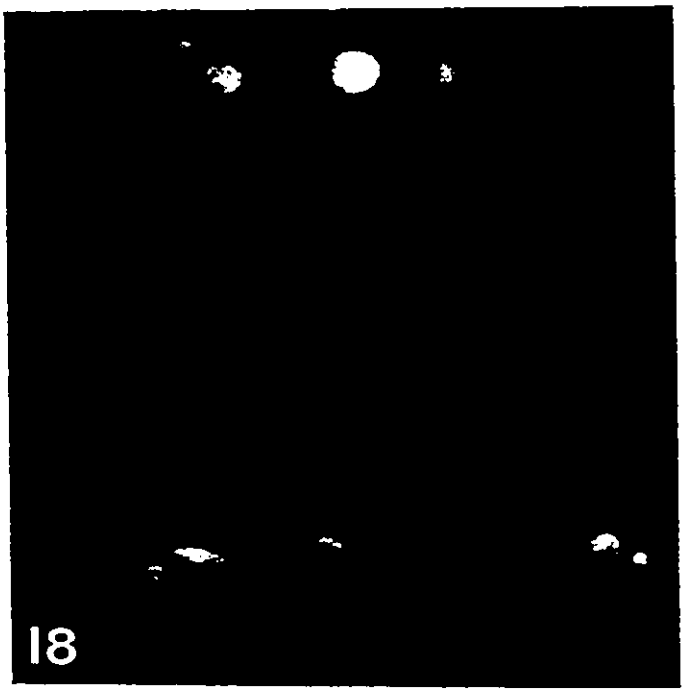




COLOURED PICTURES


16


17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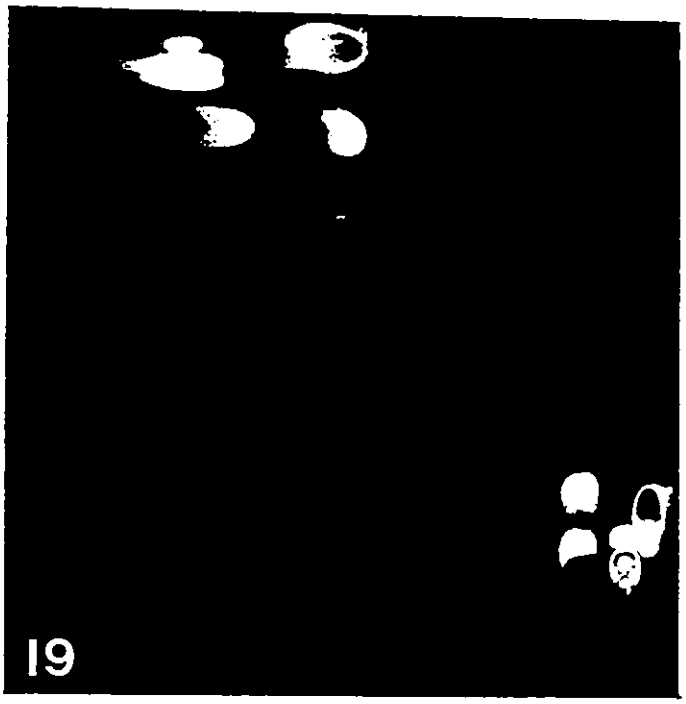

19

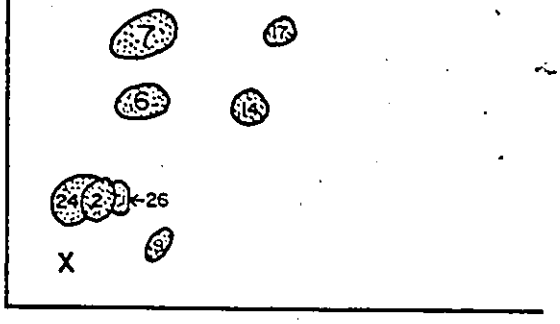
COLOURED PICTURES

The patterns indicate that as the plant matures, it diversifies the basic flavonoid pattern detected at the seedling stage. It should be noted that M. sativa, stage 3.9 and stage 4.5 have identical patterns except for the detection of compound #16 in stage 4.5 only.

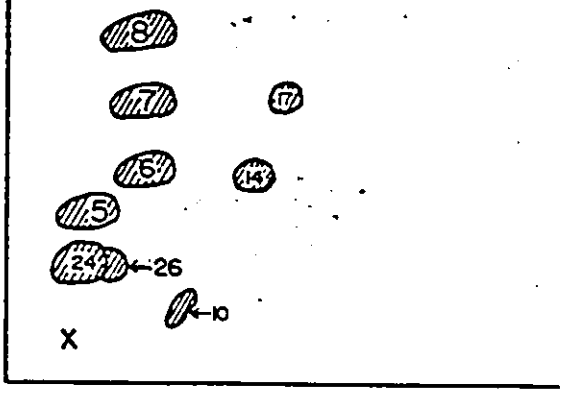
CMB=chloroform-methanol-butanone(12:2:1)
MAW=methanol-acetic acid-water(18:1:1)
X=origin

Figure 11: Spot Patterns Obtained from the Developmental Study.

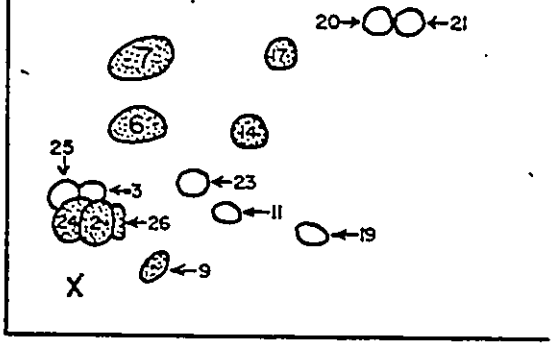
M. lupulina, seedling-stage 2.0



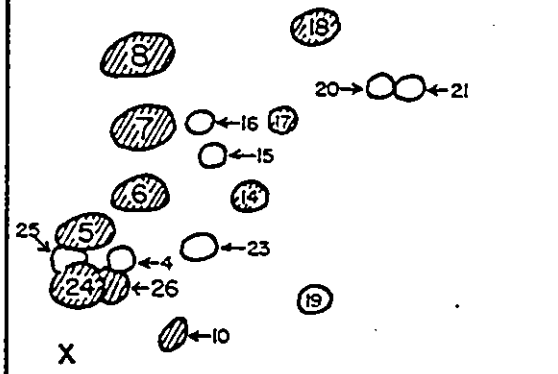
M. sativa, seedling-stage 2.0



M. lupulina, mature-stage 4.5



M. sativa, vegetative and mature-stages 3.9 and 4.5



M. arborea, vegetative and mature-stages 3.9 and 4.5

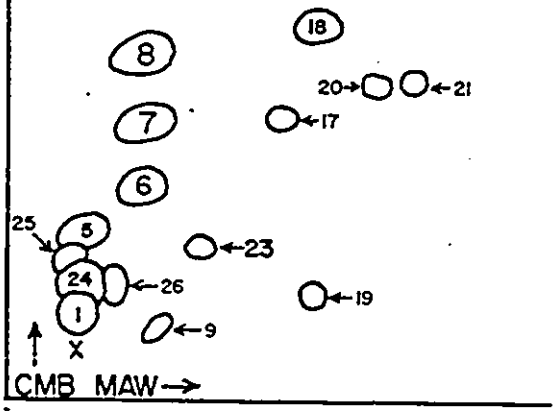
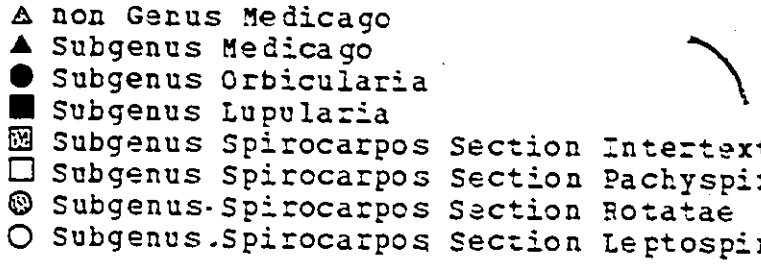


TABLE 5

Interspecific Detection of Phenolic Compounds

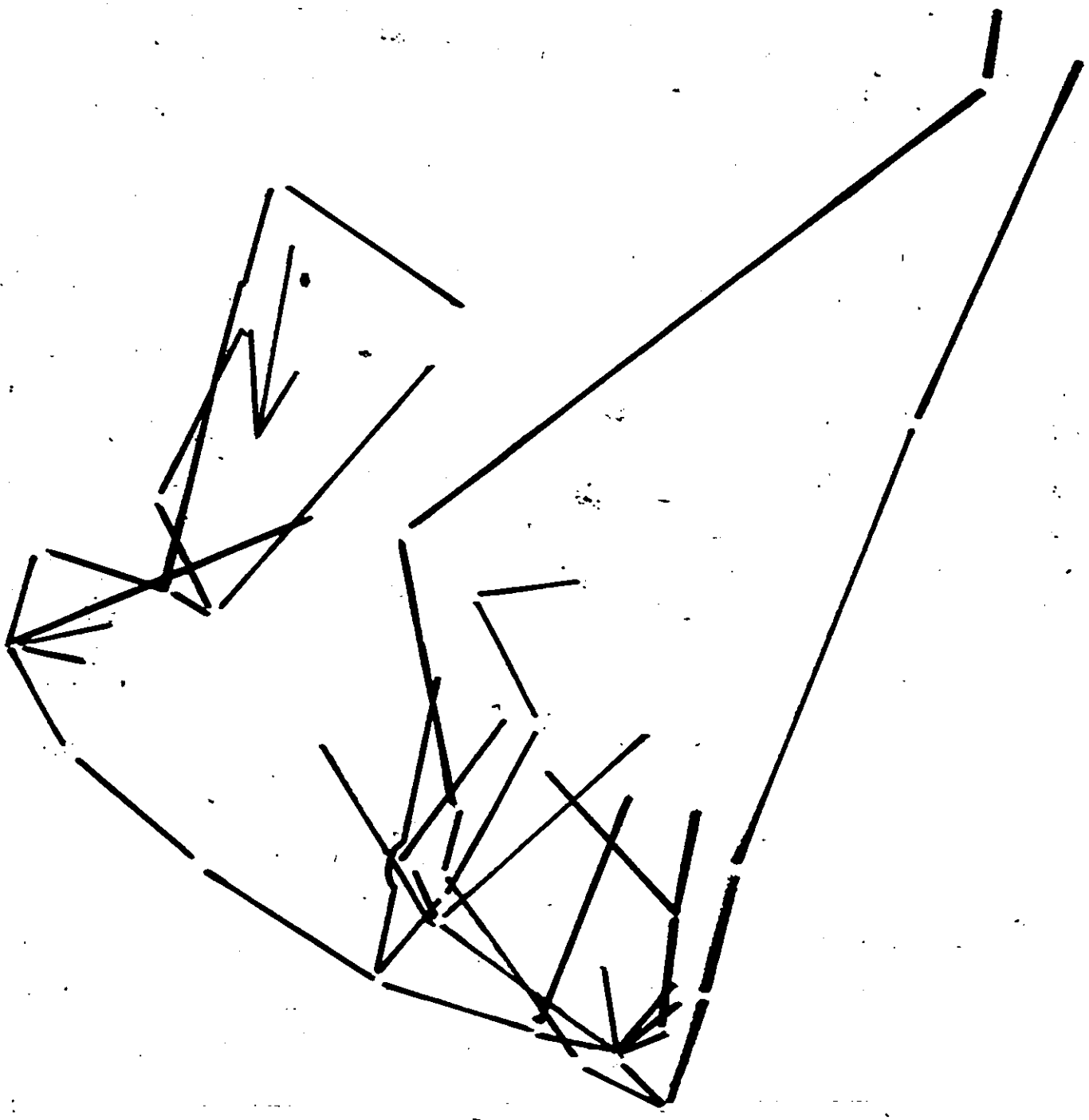
	Compound Number																											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
SATI	0	0	0	1	1	1	1	1	0	1	0	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
PROS	0	0	1	1	1	1	1	1	0	1	0	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	0
CANC	0	0	0	1	1	1	1	1	0	1	1	1	1	0	1	1	0	1	1	1	1	0	1	1	0	0	1	0
SAXA	0	0	1	0	1	1	1	1	0	1	0	0	0	0	1	0	0	1	1	0	0	0	1	1	0	1	0	0
DAGH	0	0	1	0	1	1	1	1	0	1	0	0	1	0	1	0	1	1	0	0	0	0	1	1	0	1	0	0
PIRO	0	0	0	1	1	1	1	1	0	1	0	0	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1	0
MAFI	0	0	0	0	1	1	1	1	0	1	0	0	0	0	0	0	1	1	1	1	1	0	1	1	0	0	0	0
ARBO	1	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	1	1	1	1	1	0	1	1	1	1	1	0
HYBR	1	1	0	1	0	1	1	0	0	0	0	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	0
SUFF	1	1	1	1	1	1	1	0	0	0	0	1	0	1	1	0	0	0	1	1	1	0	1	1	0	1	1	0
CRET	1	0	1	0	1	0	0	0	1	1	1	0	0	1	0	0	0	0	1	0	0	0	1	1	0	0	0	0
CAFS	1	1	1	1	0	1	0	0	1	1	1	0	1	1	1	0	1	0	1	1	1	0	1	1	1	1	1	0
RADI	1	1	1	1	0	1	1	0	1	1	1	0	1	1	1	0	1	0	0	1	1	0	1	1	0	1	1	0
OFBI	1	0	1	1	0	1	0	0	1	1	1	0	1	1	1	0	1	0	1	1	1	0	1	1	0	1	1	0
HEYN	1	0	1	1	1	1	0	0	1	1	1	0	1	1	1	0	0	0	1	1	1	0	1	1	0	0	1	0
PUBE	1	1	0	1	0	1	1	1	0	1	1	0	1	0	1	0	0	0	1	1	1	0	1	1	1	1	1	0
PLAT	1	1	1	1	0	1	1	0	1	1	1	0	1	1	1	0	1	0	0	0	0	0	1	1	0	1	1	0
RUTH	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	0	1	0	0	0	1	1	0	1	1	0
MINI	0	1	0	1	0	1	1	0	1	1	0	0	1	0	0	0	1	0	1	0	0	1	1	1	0	1	0	0
LANI	1	0	0	0	1	1	1	1	1	1	1	0	0	1	1	0	1	0	1	1	1	0	1	1	0	1	0	0
TENO	1	0	0	1	1	1	0	0	1	1	1	0	0	1	0	0	0	0	1	1	1	1	1	1	1	1	1	0
SAUV	1	0	1	1	0	1	0	0	1	1	1	0	1	1	1	0	1	0	1	1	1	0	1	1	1	1	0	0
DISC	0	0	0	1	0	1	0	0	0	1	1	0	1	1	0	0	1	0	1	0	0	0	1	1	1	1	1	0
LACI	1	1	1	1	1	1	0	0	1	1	1	0	1	1	1	0	0	0	1	1	1	0	1	1	1	1	0	0
CORO	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	0	1	1	1	1	1	0
PFAE	0	0	0	1	1	1	1	1	0	1	0	0	0	1	1	1	1	0	1	1	1	0	1	1	1	1	1	0
POLY	1	1	0	1	1	1	1	0	0	1	1	0	1	1	1	0	1	0	1	0	0	0	1	1	1	1	1	0
ARAB	1	0	0	1	0	1	1	1	0	1	0	1	0	1	1	0	0	0	1	1	1	0	1	1	1	1	1	0
SECU	0	0	1	0	0	1	1	1	0	0	0	1	0	0	0	0	1	1	1	1	1	0	1	1	1	1	1	0
LUPU	1	1	1	1	0	1	1	0	1	1	1	0	1	1	1	0	1	0	1	1	1	0	1	1	1	1	0	0
NOEA	0	0	1	1	1	1	1	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	1	0	1	0	0
CONS	0	1	0	0	0	1	1	0	0	1	0	0	1	1	0	0	1	0	1	0	0	0	1	1	0	1	0	0
RIGI	0	1	0	1	1	1	1	1	0	1	0	0	1	1	1	0	1	1	1	1	1	0	1	1	0	1	1	0
LITT	1	0	0	0	1	1	1	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	1	0
TORN	1	0	1	1	1	1	1	1	1	1	0	0	0	1	1	0	1	1	1	0	0	0	1	1	0	1	1	0
TPUN	0	0	1	1	1	1	1	1	1	0	0	0	0	1	1	0	1	1	1	0	0	0	1	1	0	1	1	0
SOLE	0	0	1	0	1	1	1	1	1	0	0	0	0	0	1	0	0	1	0	1	0	0	1	1	0	1	1	0
TUFB	0	0	1	1	1	1	1	1	1	1	0	0	1	1	1	0	1	1	0	0	0	0	1	1	0	1	1	0
MUPE	1	1	0	1	0	1	1	0	1	1	1	0	1	1	1	0	0	0	1	1	1	0	1	1	0	1	1	0
DCLI	0	1	0	1	1	1	1	1	1	1	0	0	1	1	1	0	1	0	1	1	1	0	1	1	0	1	1	0
GRAN	1	0	1	1	0	1	1	0	1	1	1	0	1	1	1	0	0	0	0	0	0	0	1	1	0	0	1	0
MUPI	0	0	1	0	0	1	1	0	0	1	0	0	0	0	1	0	1	0	1	1	1	0	1	1	0	1	0	0
INTE	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	0	1	1	1	0	1	1	0	0	0	0
BOTA	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	0	1	0	1	1	1	1	1	1	0	1	1	0
BLAN	1	1	1	1	0	1	1	0	0	1	0	1	1	1	0	0	0	0	1	0	0	0	1	1	0	1	1	0
PUGO	1	0	1	1	0	1	1	0	0	1	0	1	1	1	1	0	1	0	1	1	1	0	1	1	1	1	1	0
SCUT	0	1	1	1	0	1	1	0	0	1	0	0	1	0	1	0	0	0	1	1	1	0	1	1	1	1	1	0
MELI	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TCAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
TCOR	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0

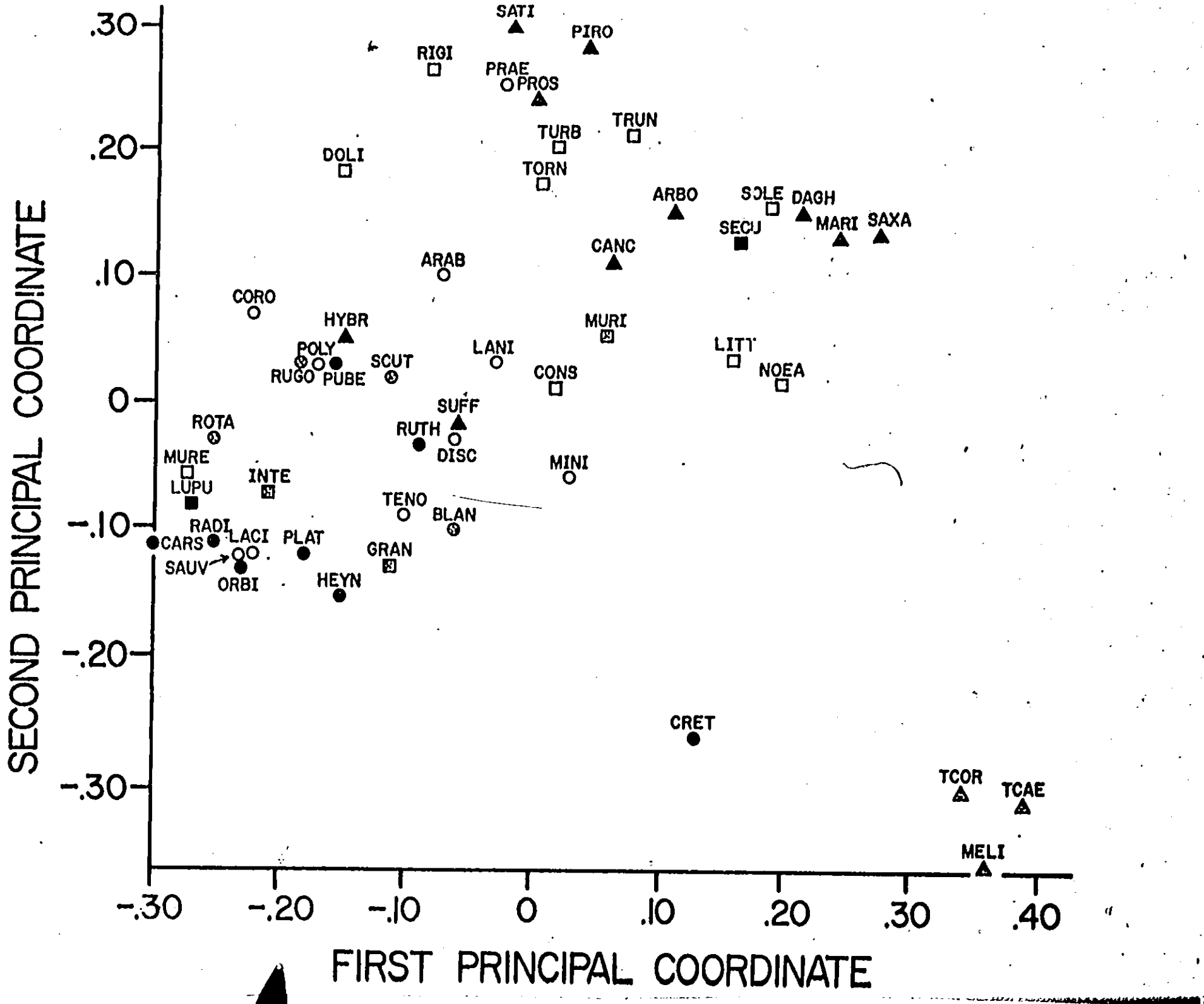
The presence of a compound is represented by a 1 and the inability to detect a compound is represented by a 0. A list of full species names is given in Appendix Table 10.

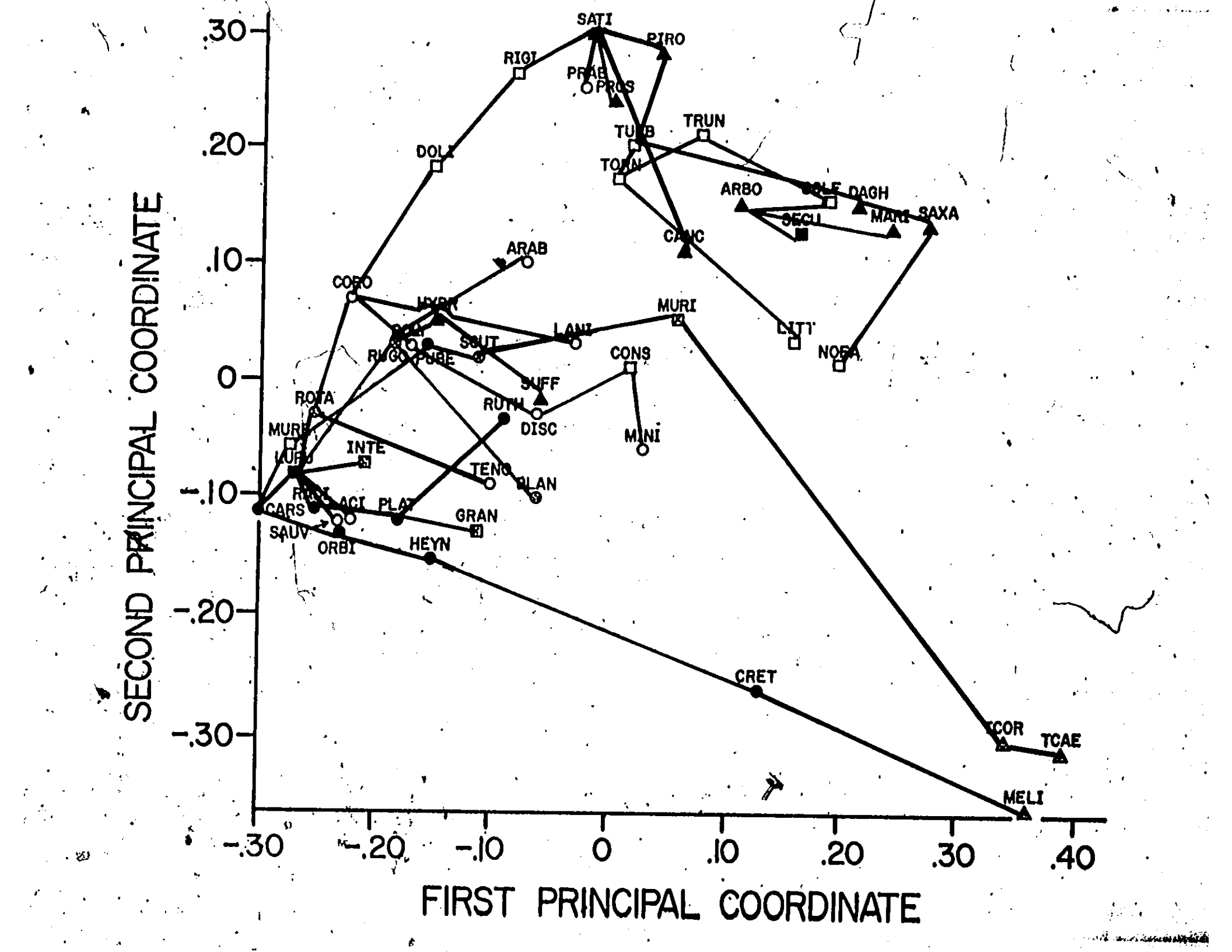


The overlay of the minimum spanning tree helps visualize any distortion. A list of full species names is given in Appendix Table 10.

Figure 12: Plot on First Two Axes of Principal Coordinate Analysis of All Species Examined.



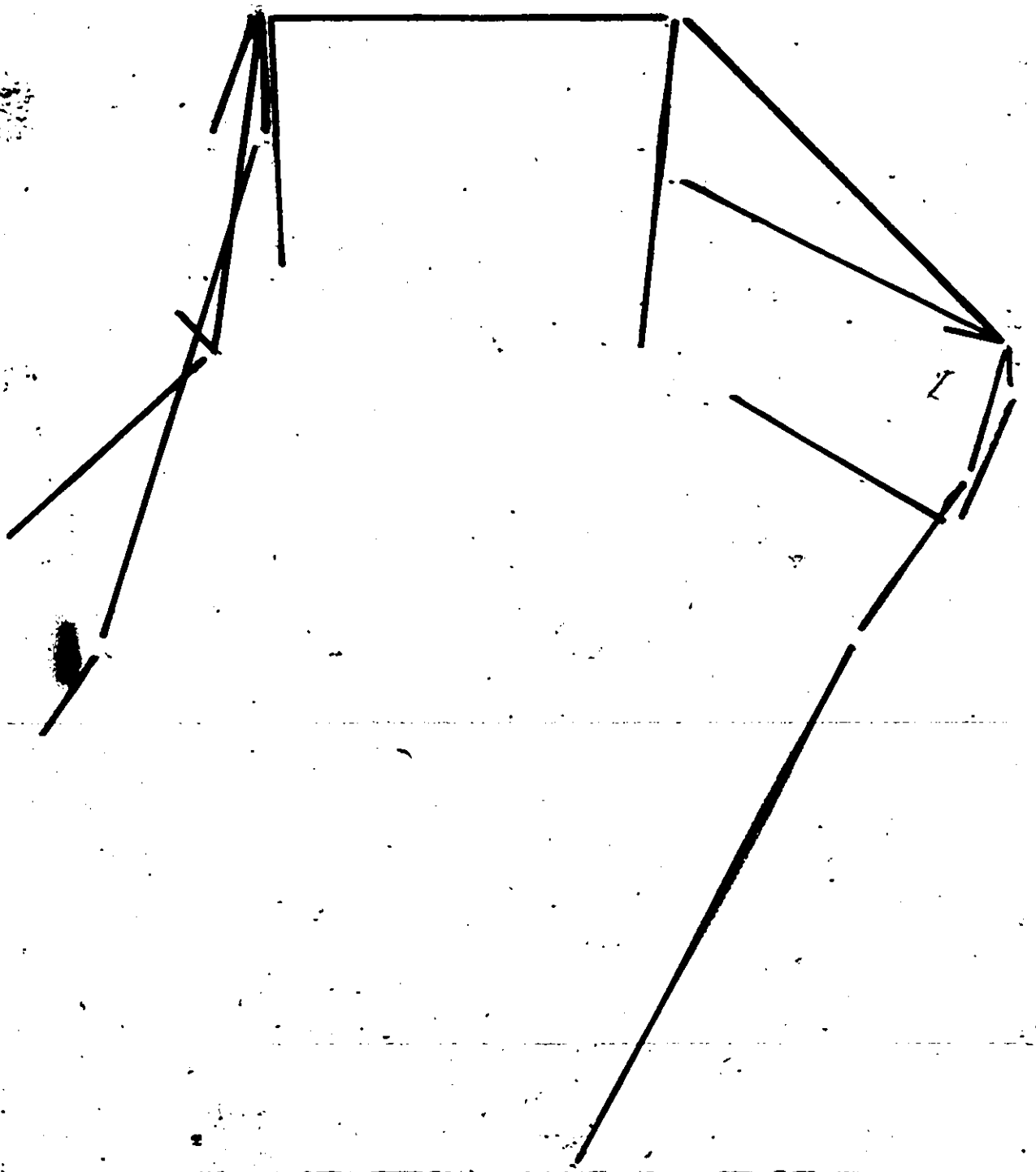

SECOND PRINCIPAL COORDINATE
FIRST PRINCIPAL COORDINATE
.30
.20
.10
0
-.10
-.20
-.30
-.30
-.20
-.10
0
.10
.20
.30
.40
SATI
PIRO
RIGI
PRAE
PROS
TRUN
TURB
DOLI
TORN
ARBO
SOLE
DAGH
SECU
MARI
SAXA
CANC
ARAB
CORO
HYBR
MURI
POLY
LANI
LITT
SCUT
CONS
NOEA
RUGO
PUBE
SUFF
ROTA
RUTH
DISC
MURE
MINI
INTE
LUPU
TENO
BLAN
RADI
CARS
LACI
PLAT
GRAN
SAUV
ORBI
HEYN
CRET
TCOR
TCAE
MELI

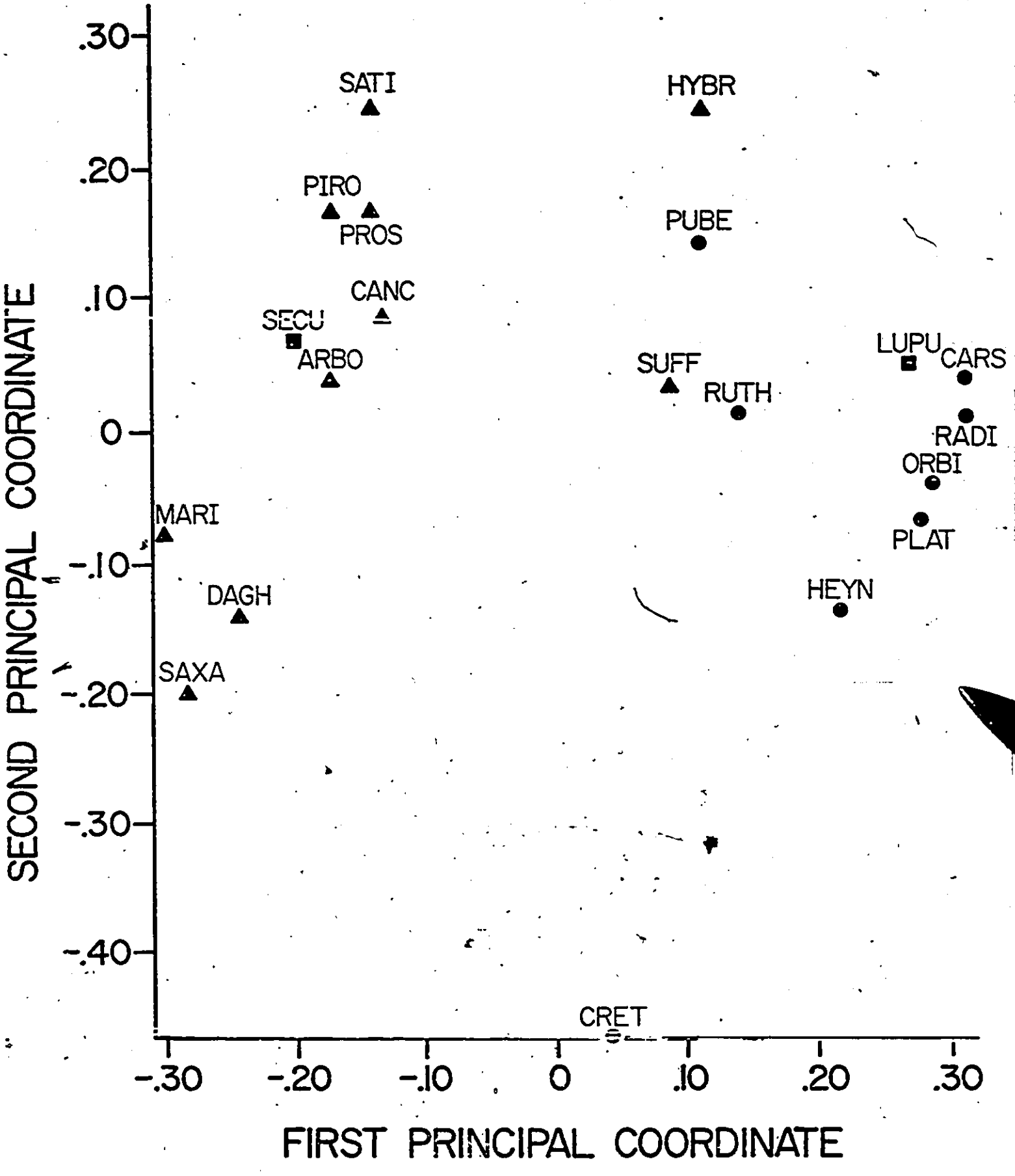

SATI
PIRO
RIGI
PRAE
PROS
TRUN
TURB
DOLI
TORN
ARBO
DAGH
SECU
MARI
SAXA
CANC
ARAB
COBO
HYBR
MURI
LANI
LITT
SOUT
NOBA
RUGO
PUBE
CONS
SUFF
ROTA
RUTH
DISC
MINI
INTE
TENO
PLAN
ACI
PLAT
GRAN
CARS
SAUV
ORBI
HEYN
CRET
TCOR
TCAE
MELI
.30
.20
.10
0
-.10
-.20
-.30
-.30
-.20
-.10
0
.10
.20
.30
.40
SECOND PRINCIPAL COORDINATE
FIRST PRINCIPAL COORDINATE

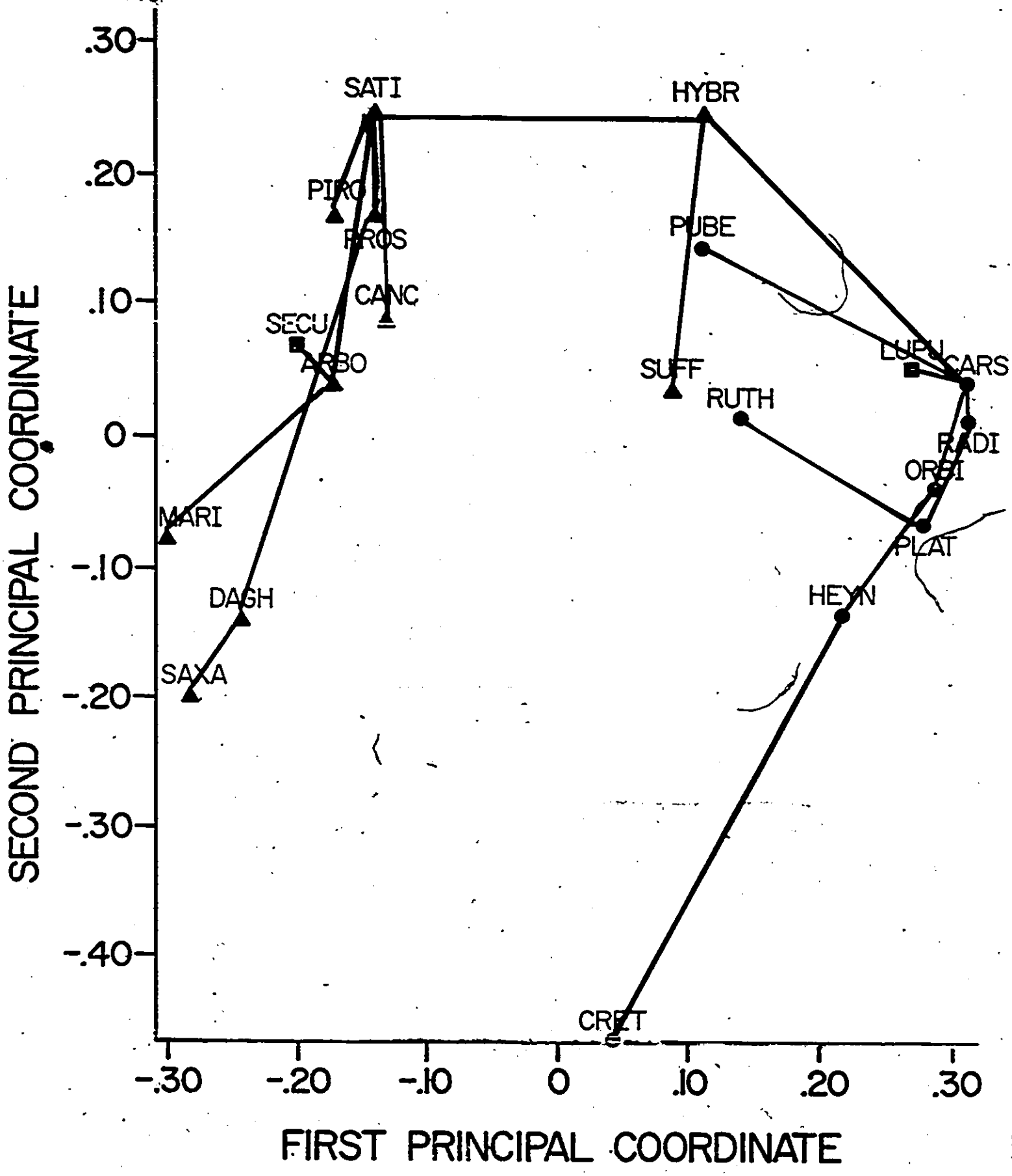
▲ Subgenus Medicago
● Subgenus Orbicularia
■ Subgenus Lupularia

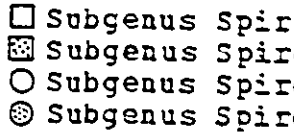
The overlay of the minimum spanning tree helps visualize any distortion. A list of full species names is given in Appendix Table 10.

Figure 13: Plot on First Two Axes of Principal Coordinate Analysis of Non-Spirocarpos Species.



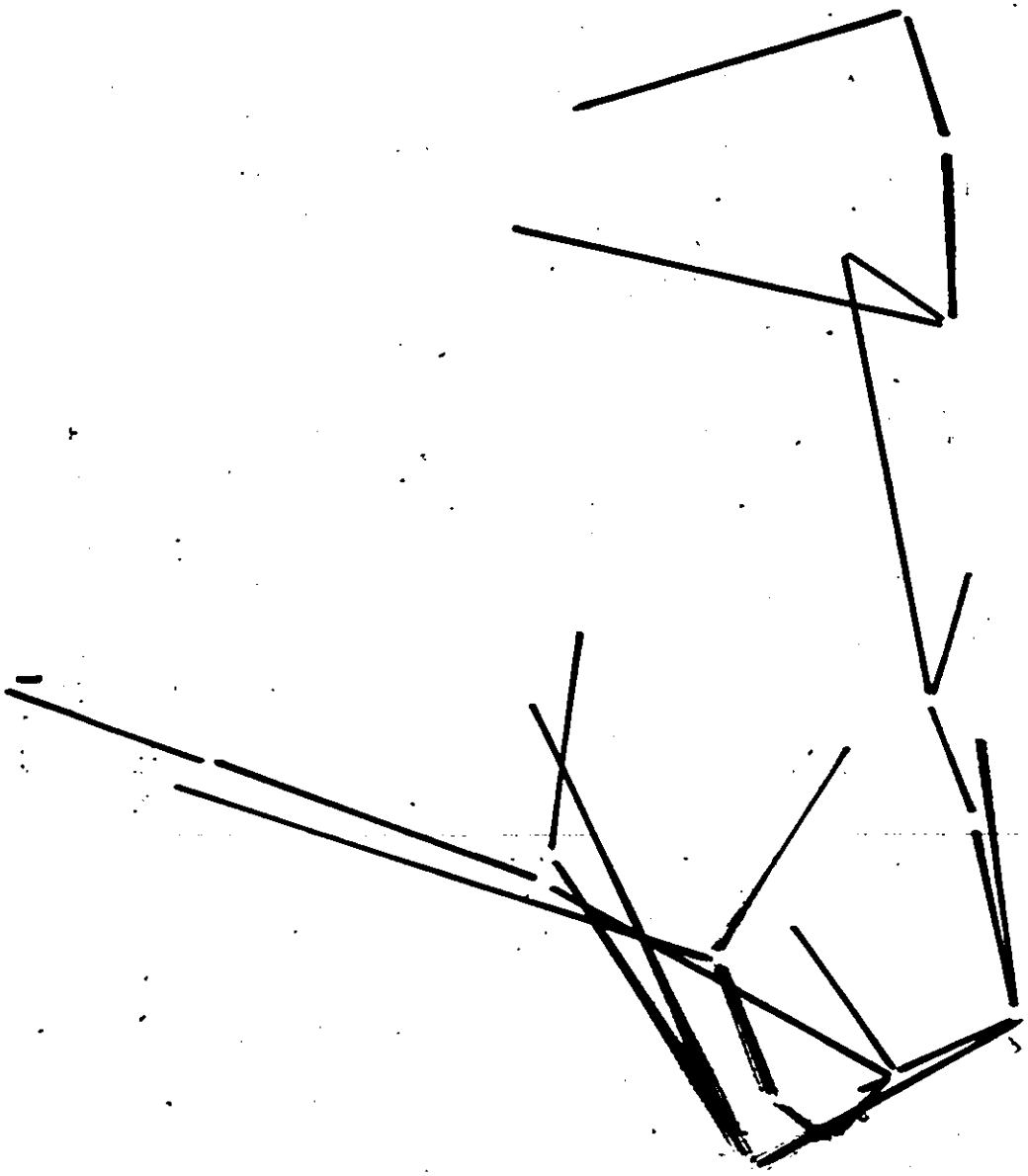

.30
.20
.10
0
-.10
-.20
-.30
-.40
SECOND PRINCIPAL COORDINATE
SATI
HYBR
PIRO
PROS
PUBE
CANC
SECU
ARBO
SUFF
RUTH
LUPU
CARS
RADI
ORBI
PLAT
MARI
DAGH
HEYN
SAXA
CRET
-.30
-.20
-.10
0
.10
.20
.30
FIRST PRINCIPAL COORDINATE

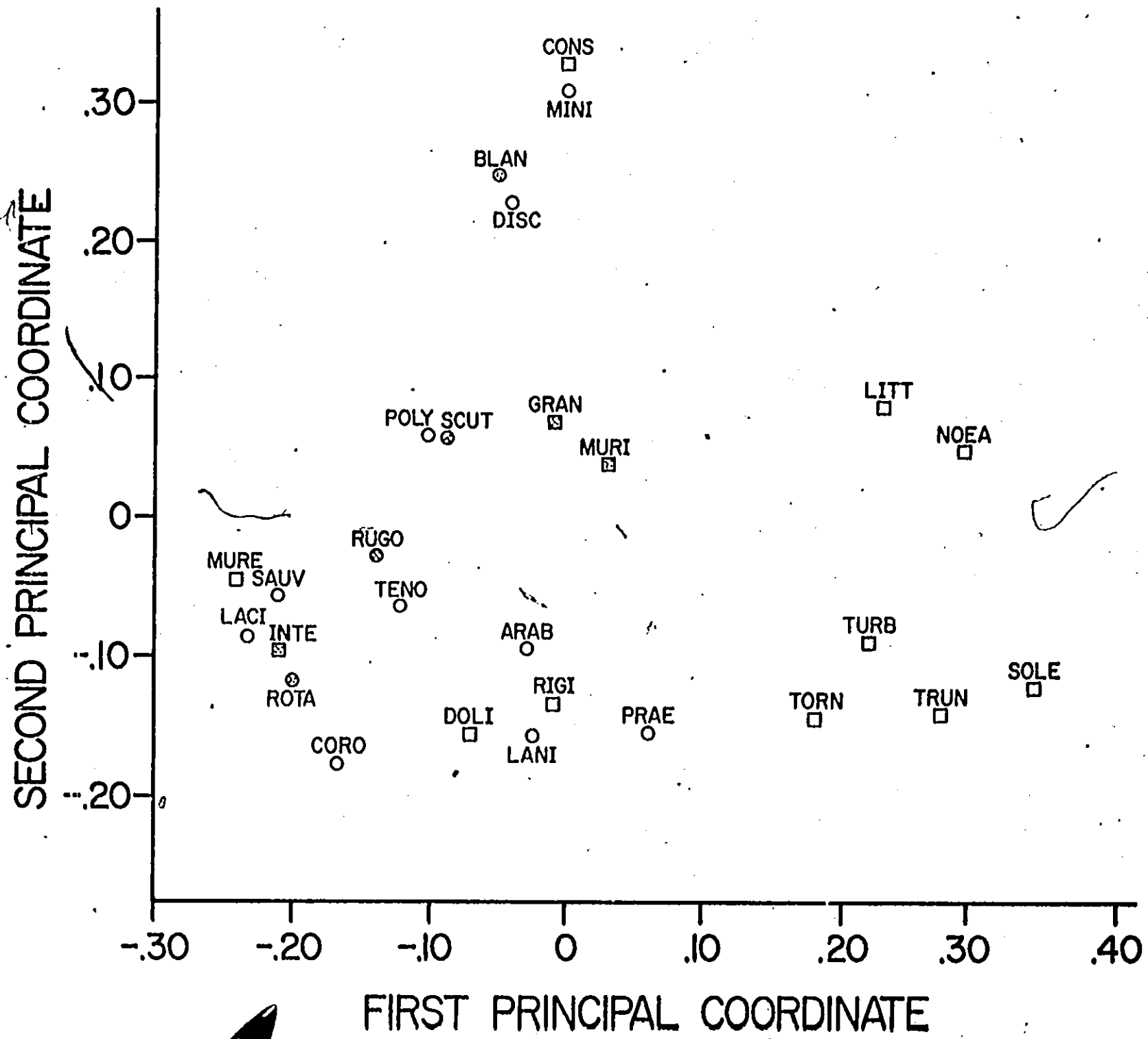

SATI
HYBR
PIRO
PUBE
BROS
CANC
SECU
LUPU
CARS
ARBO
SUFF
RUTH
RADI
ORBI
MARI
PLAT
DAGH
HEYN
SAXA
CRET
.30
.20
.10
0
-.10
-.20
-.30
-.40
-.30
-.20
-.10
0
.10
.20
.30
SECOND PRINCIPAL COORDINATE
FIRST PRINCIPAL COORDINATE

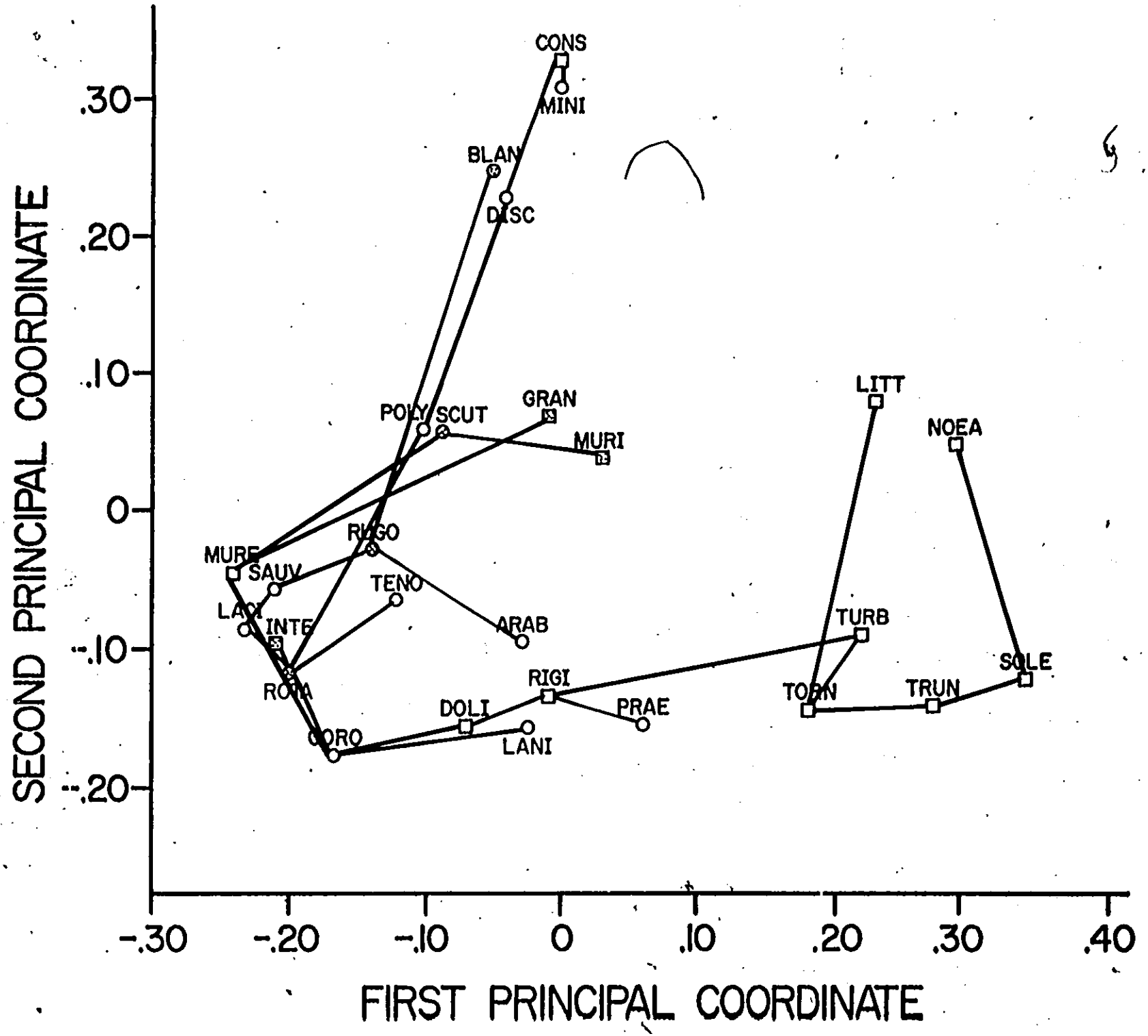


The overlay of the minimum spanning tree helps visualize any distortion. A list of full species names is given in Appendix Table 10.

Figure 14: Plot on First Two Axes of Principal Coordinate Analysis of Spirocarpos Species.



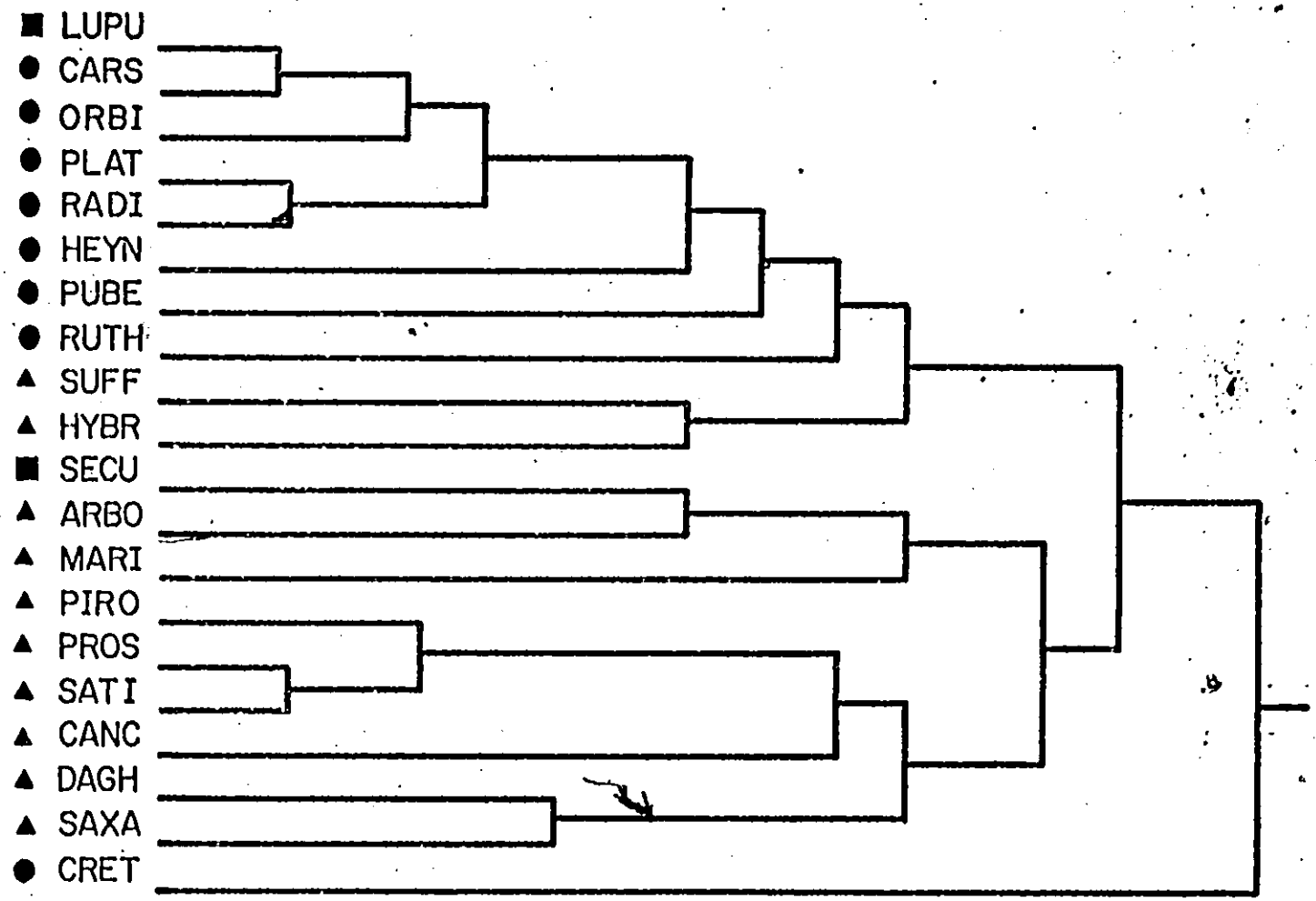

CONS
MINI
BLAN
DISC
LITT
GRAN
POLY
SCUT
NOEA
MURI
RUGO
MURE
SAUV
TENO
LACI
INTE
ARAB
TURB
SOLE
RIGI
ROTA
TORN
TRUN
DOLI
PRAE
CORO
LANI
.30
.20
.10
0
-.10
-.20
-.30
-.20
-.10
0
.10
.20
.30
.40
SECOND PRINCIPAL COORDINATE
FIRST PRINCIPAL COORDINATE


CONS
MINI
BLAN
DISC
POLY
SCUT
GRAN
MURI
RUGO
MURE
SAUV
TENO
LAGY
INTE
ARAB
ROTA
RIGI
DOLI
PRAE
ORO
LANI
LITT
NOEA
TURB
SOLE
TORN
TRUN
.30
.20
.10
0
-.10
-.20
-.30
-.20
-.10
0
.10
.20
.30
.40
SECOND PRINCIPAL COORDINATE
FIRST PRINCIPAL COORDINATE

▲ Subgenus Medicago
● Subgenus Orbicularia
■ Subgenus Lupularia

The dendrogram is the result from the average linkage clustering based on the simple matching coefficient. A list of full species names is given in Appendix Table 10.

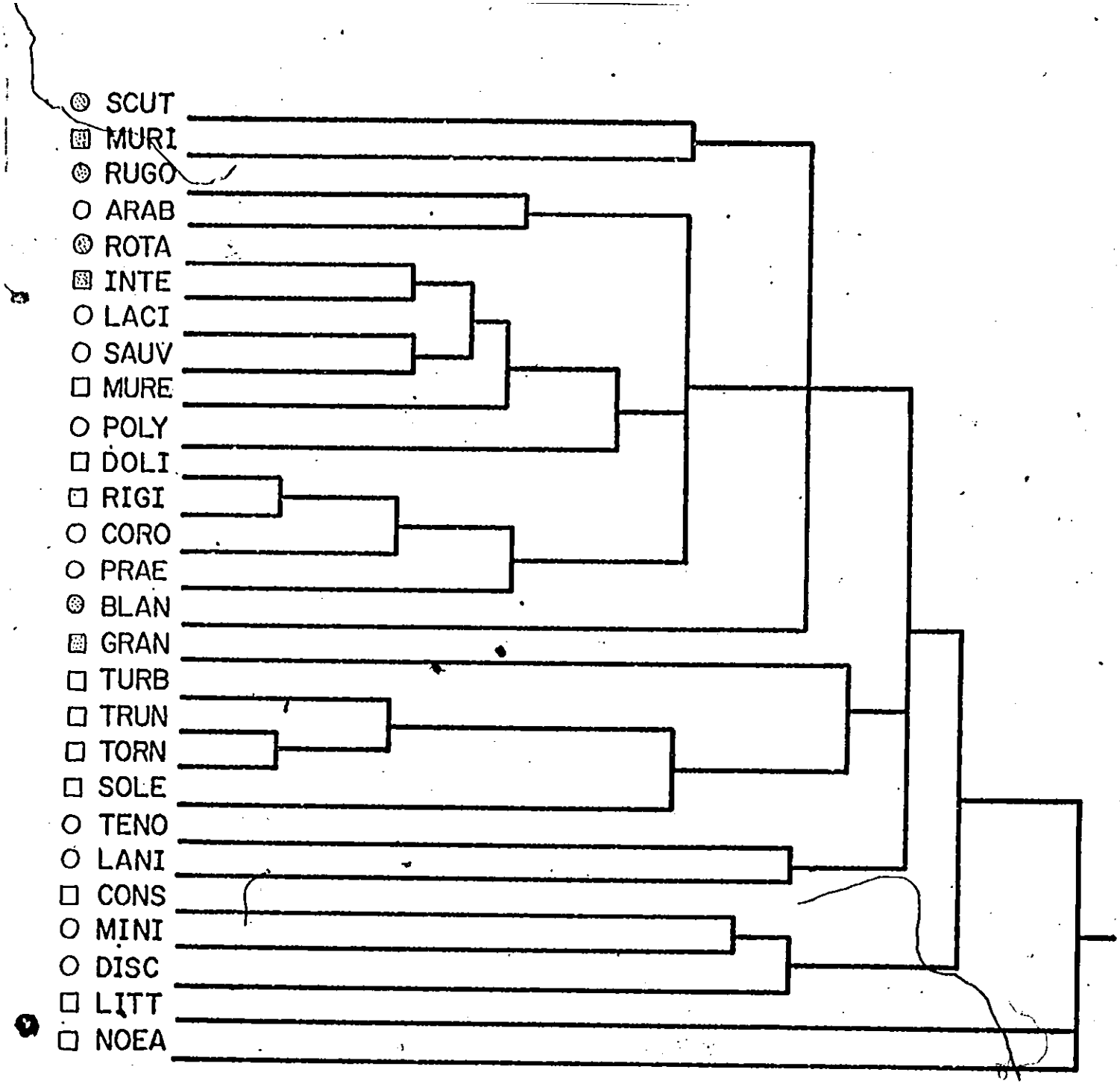
Figure 15: Dendrogram of Non-Spirocarpos Species.

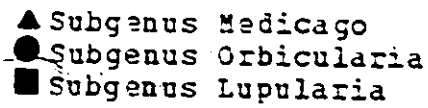

LUPU
CARS
ORBI
PLAT
RADI
HEYN
PUBE
RUTH
SUFF
HYBR
SECU
ARBO
MARI
PIRO
PROS
SATI
CANC
DAGH
SAXA
CRET

□ Subgenus Spirocarpos Section Pachyspirae
▨ Subgenus Spirocarpos Section Intertextae
○ Subgenus Spirocarpos Section Leptospirae
⊗ Subgenus Spirocarpos Section Rotatae

The dendrogram is the result from the average linkage clustering based on the simple matching coefficient. A list of full species names is given in Appendix Table 10.

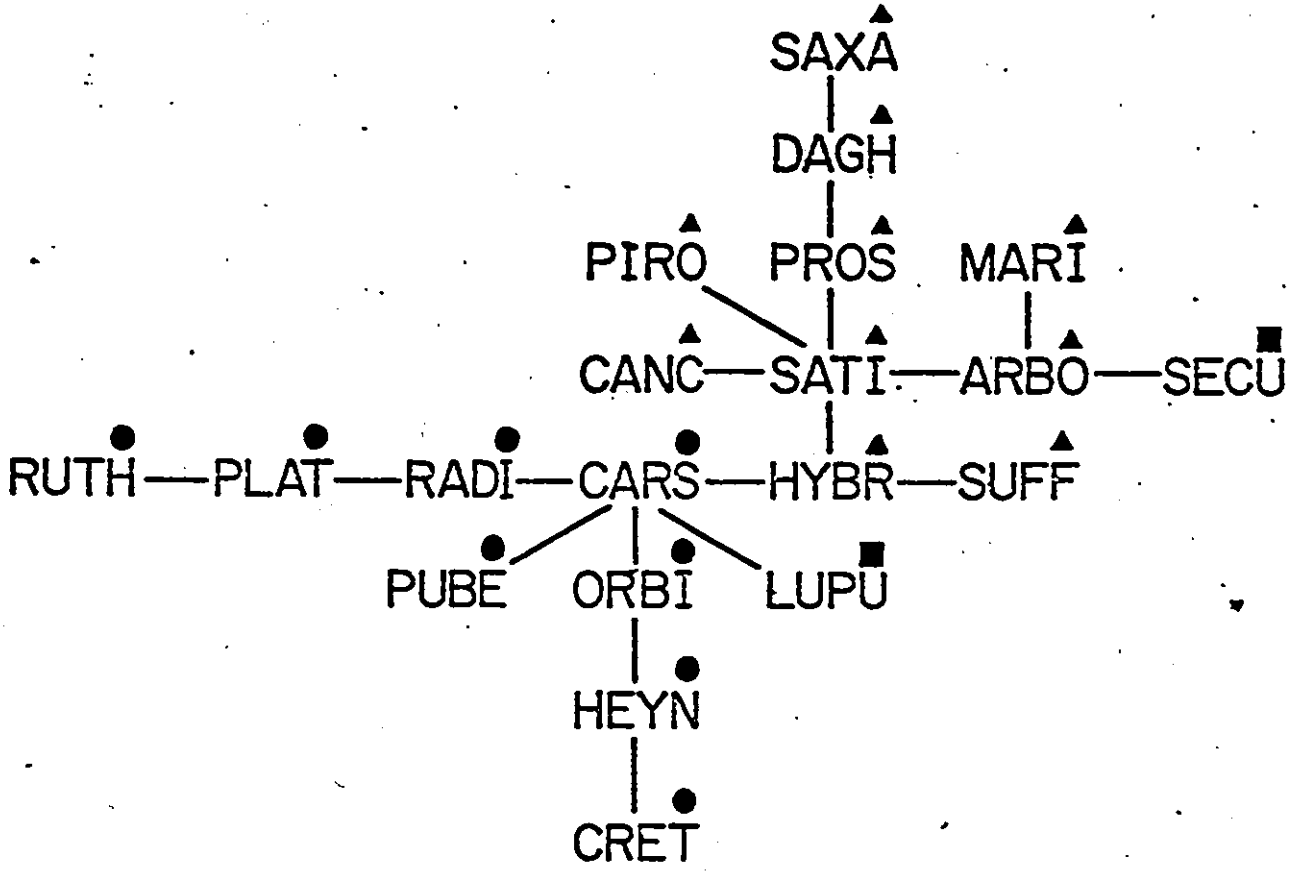
Figure 16: Dendrogram of Spirocarpos Species.

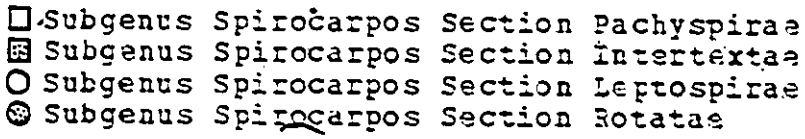

SCUT
MURI
RUGO
ARAB
ROTA
INTE
LACI
SAUV
MURE
POLY
DOLI
RIGI
CORO
PRAE
BLAN
GRAN
TURB
TRUN
TORN
SOLE
TENO
LANI
CONS
MINI
DISC
LITT
NOEA



The minimum spanning tree is based on the simple matching coefficient. The lines do not reflect relative distances, that is, the length of the lines are not proportional to the dissimilarities. A list of full species names is given in Appendix Table 10.

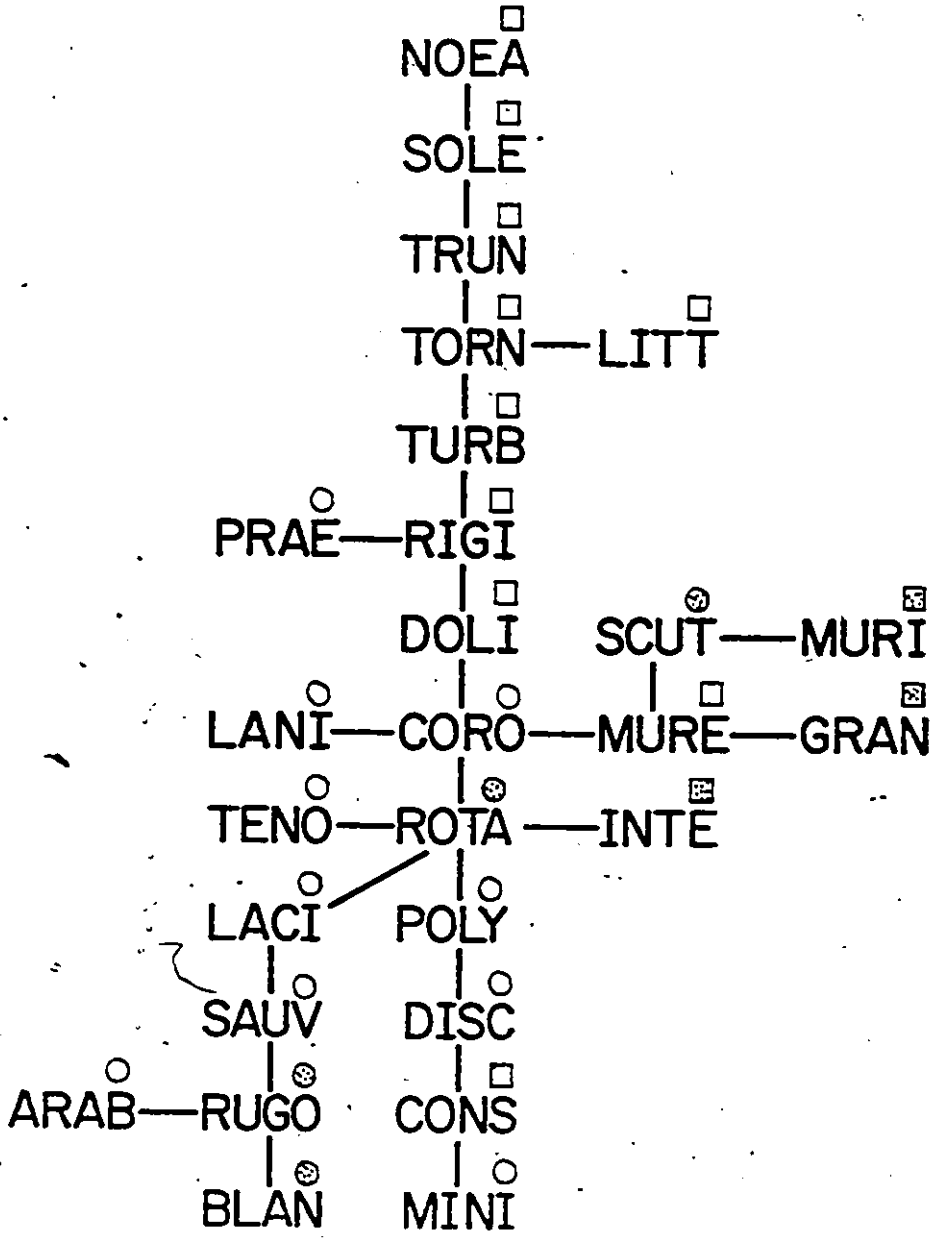
Figure 17: Minimum Spanning Tree of Non-Spirocarpos Species.


SAXA
DAGH
PIRO
PROS
MARI
CANC
SATI
ARBO
SECU
RUTH
PLAT
RADI
CARS
HYBR
SUFF
PUBE
ORBI
LUPU
HEYN
CRET



The minimum spanning tree is based on the simple matching coefficient. The lines do not reflect relative distances, that is, the length of the lines are not proportional to the dissimilarities. A list of full species names is given in Appendix Table 10.

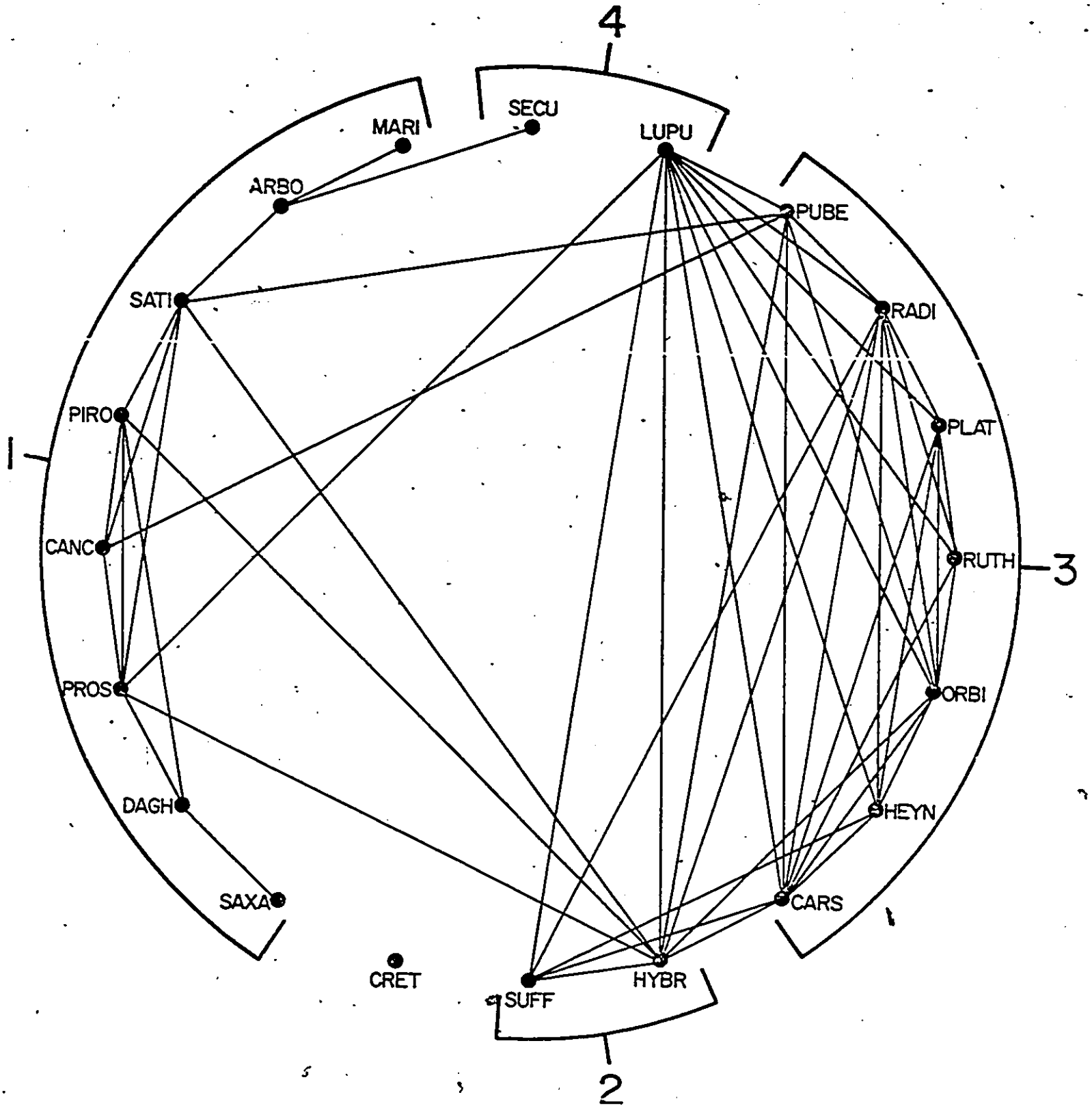
Figure 18: Minimum Spanning Tree of Spirocarpos Species.


NOEA
SOLE
TRUN
TORN
LITT
TURB
PRAE
RIGI
DOLI
SCUT
MURI
LANI
CORO
MURE
GRAN
TENO
ROTA
INTE
LACI
POLY
SAUV
DISC
ARAB
RUGO
CONS
BLAN
MINI

The graph is based on analysis using the simple matching coefficient. A line joining two species indicates that they link at a dissimilarity level no greater than 0.60. A list of full species is given in Appendix Table 10.

Group 1=Subgenus Medicago Sections Falcago, Marinae, Arboreae
Group 2=Subgenus Medicago Section Suffruticosae
Group 3=Subgenus Orbicularia
Group 4=Subgenus Lupularia

Figure 19: Maximal Connected Subgraph of Non-Spirocarpos Species.


4
SECU
MARI
LUPU
ARBO
PUBE
SATI
RADI
PIRO
PLAT
1
CANC
RUTH
3
PROS
ORBI
DAGH
HEYN
SAXA
CARS
CRET
HYBR
SUFF
2

A line joining two species indicates that they link at a dissimilarity level no greater than 0.55. A list of full species names is given in Appendix Table 10.

Group 1=Subgenus Spirocarpos Section Pachyspirae
Group 2=Subgenus Spirocarpos Section Intertextae
Group 3=Subgenus Spirocarpos Section Fotatae
Group 4=Subgenus Spirocarpos Section Leptospirae

Figure 20: Maximal Connected Subgraph of Spirocarpos Species.

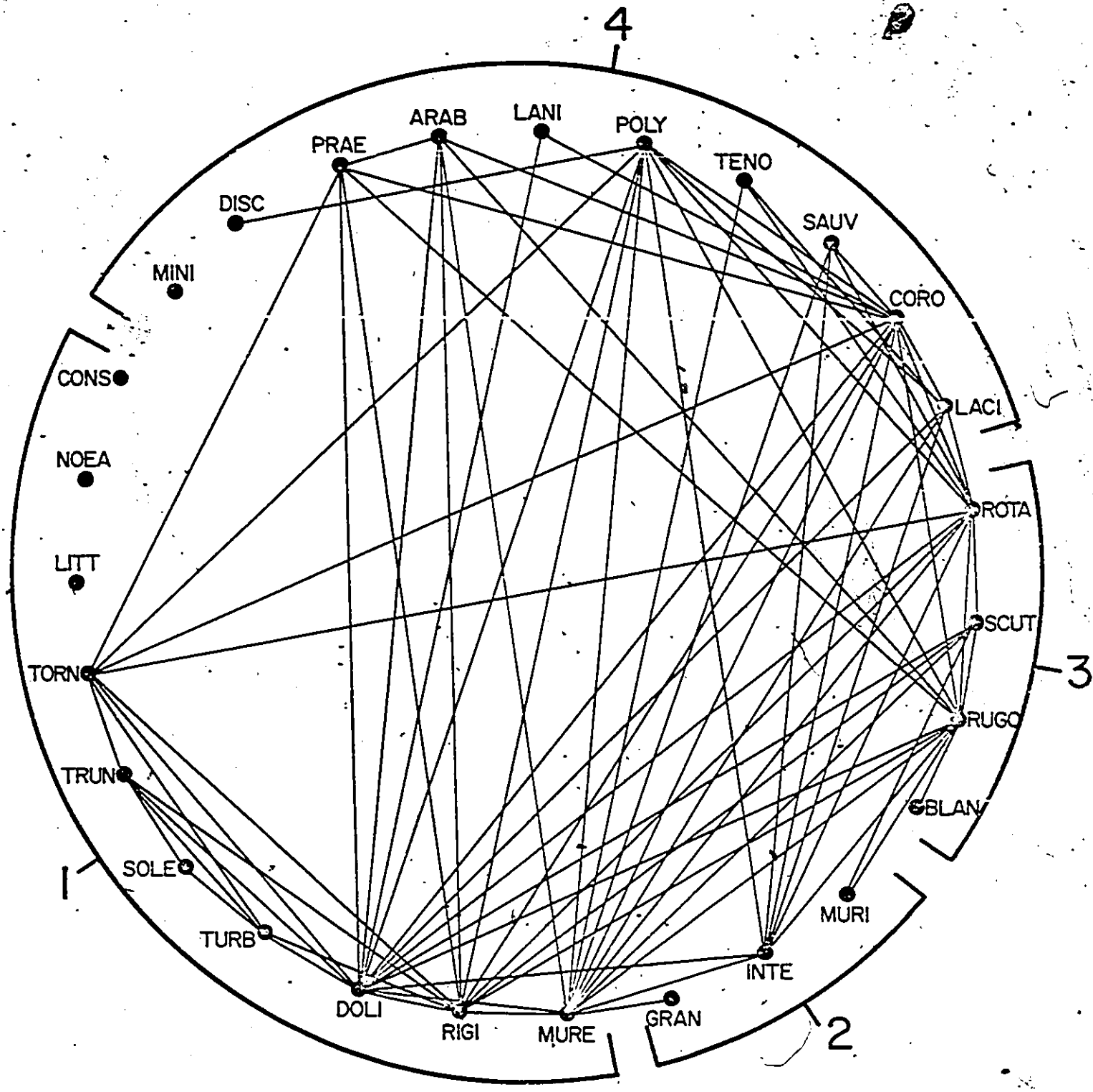

4
ARAB
LANI
POLY
PRAE
TENO
DISC
SAUV
MINI
CORO
CONS
LACI
NOEA
ROTA
LITT
SCUT
TORN
3
RUGO
TRUN
BLAN
SOLE
1
MURI
TURB
INTE
DOLI
GRAN
RIGI
MURE
2

TABLE 6

Square Distances (Gower 1971) Between Sets of Data for the 47 Species Examined

	Morphology Excluding Pollen	Vegetative Morphology	Fruit Morphology	Floral Morphology	Pollen Morphology	Flavonoid Chemistry
Number of Characters	100	27	48	26	12	26
Morphology Excluding Pollen	0.0					
Vegetative Morphology	0.337055	0.0				
Fruit Morphology	0.280448	0.639957	0.0			
Floral Morphology	0.306515	0.585776	0.617631	0.0		
Pollen Morphology	0.734128	0.766667	0.767784	0.766102	0.0	
Flavonoid Chemistry	0.483836	0.650316	0.644420	0.594686	0.820864	0.0

A score of 0.0 indicates perfect agreement. A score of 1.0 indicates no aggreement whatsoever. The flavonoid data is from this study. The morphological data are from Small (1981), Small et al. (1981a,b).

TABLE 7

Compounds in the Major Taxa

	* of species	Compound Number 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Genus Medicago																		
Subgenus Medicago																		
Section Falcago	6	0	0	3	4	6	6	6	6	0	6	1	1	5	3	6	4	4
Section Arboreae	1	1	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	1
Section Marinae	1	0	0	0	0	1	1	1	1	0	1	0	0	0	0	0	0	1
Section Suffruticosae	2	2	2	1	2	1	2	2	0	0	0	0	2	1	2	2	1	1
Subgenus Orbicularia																		
Section Carstiensae	1	1	1	1	1	0	1	0	0	1	1	1	0	1	1	1	0	1
Section Platycarpae	2	2	2	2	2	0	2	2	1	2	2	2	1	2	1	2	1	2
Section Orbiculares	1	1	0	1	1	0	1	0	0	1	1	1	0	1	1	1	0	1
Section Hymenocarpos	1	1	1	1	1	0	1	1	0	1	1	1	0	1	1	1	0	1
Section Heynianae	1	1	0	1	1	1	1	0	0	1	1	1	0	1	1	1	0	0
Section Cretacae	1	1	0	1	0	1	0	0	0	1	1	1	0	0	1	0	0	0
M. pubescens	1	1	1	0	1	0	1	1	1	0	1	1	0	1	0	1	0	0
Subgenus Lupularia	2	1	1	2	1	0	2	2	1	1	1	1	1	1	1	1	0	2
Subgenus Spirocarpos																		
Section Rotatae	4	3	3	4	4	1	4	4	0	1	4	1	2	4	3	3	0	2
Section Intertextae	3	2	1	3	2	1	3	3	0	2	3	2	1	2	2	3	0	2
Section Pachyspirae	10	3	4	5	7	8	10	10	8	6	7	2	0	5	8	8	0	6
Section Leptospirae	10	7	4	2	9	6	10	6	4	6	10	7	2	6	9	7	1	7
Genus Melilotus	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Genus Trigonella	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

The numbers indicate the number of species in that Section possessing that

1 OF

TABLE 7

ids in the Major Taxa

# of species	Compound Number 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
6	0	0	3	4	6	6	6	6	0	6	1	1	5	3	6	4	4	6	5	3	3	0	6	6	3	5	3	0
1	1	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	1	1	1	1	1	0	1	1	1	1	1	0
1	0	0	0	0	1	1	1	1	0	1	0	0	0	0	0	0	1	1	1	1	1	0	1	1	0	0	0	0
2	2	2	1	2	1	2	2	0	0	0	0	2	1	2	2	1	1	0	2	2	2	0	2	2	1	2	2	0
1	1	1	1	1	0	1	0	0	1	1	1	0	1	1	1	0	1	0	1	1	1	0	1	1	1	1	1	0
2	2	2	2	2	0	2	2	1	2	2	2	1	2	1	2	1	2	0	1	0	0	0	1	1	0	1	1	0
1	1	0	1	1	0	1	0	0	1	1	1	0	1	1	1	0	1	0	1	1	1	0	1	1	0	1	1	0
1	1	1	1	1	0	1	1	0	1	1	1	0	1	1	1	0	1	0	0	1	1	0	1	1	0	1	1	0
1	1	0	1	1	1	1	0	0	1	1	1	0	1	1	1	0	0	0	1	1	1	0	1	1	0	0	1	0
1	1	0	1	0	1	0	0	0	1	1	1	0	0	1	0	0	0	0	1	0	0	0	1	1	0	0	0	0
1	1	1	0	1	0	1	1	1	0	1	1	0	1	0	1	0	0	0	1	1	1	0	1	1	1	1	1	0
2	1	1	2	1	0	2	2	1	1	1	1	1	1	1	1	1	0	2	1	2	2	2	0	2	2	2	2	1	0
4	3	3	4	4	1	4	4	0	1	4	1	2	4	3	3	0	2	0	4	3	3	1	4	4	2	4	4	0
3	2	1	3	2	1	3	3	0	2	3	2	1	2	2	3	0	2	0	2	2	2	0	3	3	0	1	1	0
10	3	4	5	7	8	10	10	8	6	7	2	0	5	8	8	0	6	5	6	4	3	0	10	10	0	9	8	0
10	7	4	2	9	6	10	6	4	6	10	7	2	6	9	7	1	7	0	10	7	7	2	10	10	8	10	7	0
1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0

e number of species in that Section possessing that compound.

2 of 2

1555759

Section IV

DISCUSSION

The use of polyamide microlayers and the flavone reagent provide a good technique for the study of flavonoids in Medicago. Paper and column chromatography are also practical. The compounds detected using these techniques are of value in the study of the taxonomy of the genus.

Scoring for the compounds presents a few problems. There is difficulty in scoring for the absence of a flavonoid. Presence is clear enough but to distinguish between presence in trace amounts and complete absence is difficult. Sometimes this problem can be eliminated by using chemical characters that replace one another. Flavonols and flavones can act as replacement characters with the general trend in leaf tissue being the replacement of flavonols by flavones. However, the two classes of flavonoids are not mutually exclusive and as in the case of Medicago both occurred in most species (Harborne 1975).

Using spot counting data without identifying the compounds can lead to problems as well. Often it causes an overestimation of the number of compounds because it is difficult to be sure if the same compound always occupies the

same position in the chromatograms. This would lead to errors in calculating the taxonomic distance between species. Also, extra spots may be present that are artifacts of the extraction procedure. For example, glycosides may be partially broken down into possibly a number of intermediates as well as the aglycone so what is actually a single compound may appear as three or more in 2- dimensional chromatograms (Harborne 1975). In the present study, this problem was reduced by hydrolysing the tissue for a long period of time in hopes that only aglycones are present. If, however C-glycosides are present in the tissue then another problem arises, that of isomerisation products. C-glycosides would appear as two spots (as the 6-glycoside and as the 8-glycoside) and again lead to an overestimation in number of compounds. An underestimation in the number of compounds is also possible as two or three compounds may occupy one site. For the above reasons, it is always best to try and identify at least the major or the more variable constituents (Harborne 1975).

Even the seedlings of the Medicago species are well endowed with flavonoids. As the plant matures it adds new compounds to the original pattern of compounds. It therefore seems plausible that the compounds in the seedling occur early in the biosynthetic pathway and those that appear only in the mature plant are farther along the pathway.

Some of the compounds appear to show interdependence for their occurrence. For instance, at least two series seem to be present #6→#7→#8→#18 and #26→#19→#25→#20→#21. The developmental results do not contradict this scheme. The cotyledons of both M. lūpulina and M. sativa seedlings possess compound #26 (although it is not detected in the first unifoliate leaf of either species). In the mature plants, #19, #25, #20 and #21 are present in addition to #26 in both species. It is interesting to note that at the seedling stage, #2 in M. lupulina and #5 in M. sativa are found only in the first unifoliate leaf and not in the cotyledons.

When suggesting possible biosynthetic pathways, it should be kept in mind that intermediates may be necessary but because they are rapidly used and do not accumulate, they may go undetected, or the intermediates may remain attached to enzymes and not be extracted. Since the late vegetative stage (3.9) differs little or not at all from the flowering and fruiting stage (4.5) then the diversification in the flavonoids that takes place must occur rather rapidly during the earlier vegetative period of growth.

More work is needed to identify all the compounds and to sample many more intermediate vegetative stages to determine the pathways of production. It would also be interesting to sample senescing tissues to see if compounds disappear with age.

The developmental results indicate that it was for the most part valid to compare stage 4.5 material (flowering and fruiting) which was collected for most species, to the stage 3.9 material (vegetative with approximately 9 branches). It is possible though that compounds produced at points far along the biosynthetic pathway may not be detected in stage 3.9 plants but would accumulate in stage 4.5 plants and so become detectable. However, the results do not indicate any qualitative flavonoid variation between the two stages. Stage 3.9 had to be collected for two Medicago species, M. carstiensis and M. tenoreana as well as for the two Trigonella species examined because no flowering occurred.

In the present study characters #27 and #28 correspond to cyanidin and proanthocyanidin respectively. The results reveal the widespread presence of cyanidin across the whole Medicago genus and the absence of proanthocyanidins in leaf tissue. Simon (1967) reported that cyanidin occurrs in all Subgenera except Subgenus Medicago and Subgenus Lupularia and when it did occur he indicated that it was often due to the hydrolysis of proanthocyanidins.

Proanthocyanidins are reported to occur commonly but not universally in Leguminosae (Gibbs 1974). If present in the leaves, the proanthocyanidins are also almost always present in the seeds but the reverse is not necessarily true (Bate-Smith and Ribereau-Gayon 1959). For example, the

seeds of M. lupulina are reported as positive for procyanidin (Bate-Smith and Ribereau-Gayon 1959) but as negative for proanthocyanidins in the leaves (Bate-Smith and Lerner 1954).

Simon (1967) differentiated the origin of cyanidin (either from anthocyanins or proanthocyanidins) on the basis of the colour of the leaf before extraction. He stated that if the leaf had visible red spots or stripes then the cyanidin that occurred was due to anthocyanins but if the leaf was entirely green and cyanidin still appeared then he assumed it was due to the presence of proanthocyanidins. Anthocyanins may occur in green tissue but have their colour obscured by other pigments (Harborne 1975). In order to determine the presence of proanthocyanidins, the tissues must first be suitably extracted and tested for anthocyanins. If they are present, the anthocyanins must be removed before the test for proanthocyanidins is performed. By definition, all colourless materials isolated from plants which form anthocyanidins when heated with acid are proanthocyanidins (Freudenberg and Weinges 1960). Therefore, only if an anthocyanidin-free extract turns red after being boiled in acid can it be said that proanthocyanidins occur.

The methods used in this study did not detect proanthocyanidins in the leaf tissue of any of the Medicago, Melilotus or Trigonella species examined. However anthocyanidins are widespread and, as was noted by Simon (1967), the agly-

cone is cyanidin. This study must disagree, however, with a number of the findings of Simon even if it is assumed that what he reported as proanthocyanidins were actually attributed to anthocyanidins. The present study detected anthocyanidins in a number of species of the Subgenus Medicago including M. sativa, M. cancellata, M. pironae, M. arborea, M. hybrida and M. suffruticosa which Simon reported to be totally lacking in these flavonoids. The differences are probably due to populational variation since the presence or absence of anthocyanins is determined by a single locus [with possession of recessive "c" alleles yielding acyanic plants(Barnes 1966)]. Differing techniques would affect the detection of anthocyanins, if present.

The populations of this study were grown under similar conditions and variation still is evident(Table 4). Since the same populations were not used in the two studies, the Simon work and this work, some differences could be expected. It is interesting to note, however, that Simon also found his populations of M. intertexta and M. scutellata to be highly variable. Also since plants were grown under field conditions in two very different environments (field conditions in Australia in the case of Simon and field conditions in Canada in the case of this study) then the differing conditions could also be expected to contribute to concentration differences(Singh and Thompson 1961, Alston and Turner 1963, Buttery and Buzzell 1973, Stathem and Crow-

den 1974) and, therefore, to difficulties in detecting some compounds.

In this study quercetin, kaempferol and coumestrol as well as a number of other compounds were tentatively identified. In addition to the 26 compounds indicated in the master chromatogram, other compounds were detected but these compounds were difficult to detect, or were found in perhaps one or two species and were considered unreliable. There were probably no more than five compounds that were occasionally detected but not used as characters. It seems, therefore, that many of the 53 compounds recorded by Simon and Goodall(1968) probably were unhydrolysed or only partially hydrolysed compounds).

Simon and Goodall(1968) reported that quercetin occurred all through the genus Medicago and, while the present study finds quercetin to be widespread, it is apparently lacking from the Section Falcago and Section Marinae of the Subgenus Medicago. It occurs in some species, but not in all of the remaining Subgenera.

Simon(1967) reported kaempferol as absent from the Subgenus Medicago and Subgenus Spirocarpos Sections Pachyspirae and Rotatae. This study finds it in at least some members of all the Subgenera.

Simon (1967) reported coumestrol as present in all Medicago species he studied except in one population of M. orbicularis and not at all in M. lupulina. He stated that the concentration varied widely and this study also finds this to be so. However, this study finds coumestrol to be absent from Subgenus Spirocarpos Section Pachyspirae and Section Intertextae. It is present in Subgenus Lupularia and scattered through the other Sections.

In addition to the flavonols quercetin and kaempferol, Harborne (1971) also reported the flavones luteolin and apigenin in some species. The present study finds apigenin in all Medicago species except M. cretacea and luteolin throughout the genus except in Subgenus Medicago Section Falcago. The flavones chrysoeriol and tricin were also detected.

Isoflavone accumulation was reported to be absent from Medicago (Harborne 1969). Guggolz et al. (1961) studied the presence of daidzein, formononetin, genistein and biochanin A in M. sativa. They reported the presence of daidzein, genistein and biochanin A in 1 ppm, 1 ppm and 1-5 ppm respectively which is less than 10 ppm which was the limit of detection for the technique used in the present study. Standards of genistein and biochanin A were available but no similar compounds were detected in any of the Medicago species.

The taxonomic structure suggested by the flavonoid chemistry supports the taxonomic groupings using traditional characters for the Subgenus Medicago and the Subgenus Orbicularia (refer to Table 1). The analysis of phenolics by Simon and Goodall (1968) also reported that the perennials showed strong affinities. M. arborea belongs in with the Subgenus Medicago not only as indicated by the present flavonoid study but also from the serological data of Simon (1979), and from the phytoalexin data of Ingham (1979). The latter study revealed that M. arborea produced medicarpin and vestitol which were the phytoalexins characteristic of Medicago species. The positioning of M. arborea in Subgenus Medicago was not acceptable to Small (1981).

The chaining effect of species of Subgenus Orbicularia in the average linkage dendrogram of non- Spirocarpos species supports the concept that each species in this group is very different from the others and is correctly placed in its own, monotypic Section (refer to Table 1). The minimum spanning tree also places M. ruthenica and M. platycarpa close as does the morphology. This does not contradict the classifying of the two species together in one Section, the Section Platycarpeae. A phytoalexin study by Ingham and Harborne (1976) showed that M. platycarpa belonged in Medicago as did M. radiata. The flavonoid results also support the positioning of these two controversial species in Medicago.

M. suffruticosa and M. hybrida are considered as links between Subgenus Orbicularia and Subgenus Medicago. The flavonoid chemistry supports this concept but these two transitional species appear closer to Subgenus Orbicularia than the Subgenus Medicago. Some of the Subgenus Orbicularia species are obviously morphologically related to Trigonella (Small 1981) and M. hybrida has often been placed in Trigonella rather than in Medicago itself but the flavonoid chemistry indicates that it belongs with Medicago.

The structure of the Subgenus Lupularia consisting of M. lupulina and M. secundiflora is not upheld by the flavonoid chemistry. The phytoalexin study of Ingham (1979) indicates that M. lupulina is significantly different from most other Medicago and Melilotus species. The Melilotus species accumulates only medicarpin and as mentioned above, Medicago species accumulate medicarpin and vestitol and/or sativan, however M. lupulina accumulates none of these. Heyn (1963) considered M. lupulina as doubtfully located in Medicago and pollen studies by Small et al. (1981a) indicate that the pollen morphology of M. lupulina and M. secundiflora are divergent.

The flavonoid results do not corroborate the traditional Sections of Subgenus Spirocarpos (refer to Table 1) although the chemistry does separate Subgenus Spirocarpos from the other Subgenera to a limited degree. The Section Pachyspi-

rae, however, does show closer relationships within it than do the Sections Leptospirae, Rotatae and Intertextae. The results of Simon and Goodall(1968) also suggested that the Pachyspirae are chemically distinctive.

Serological data of Simon(1979) has the Section Pachyspirae members M. truncatula, M. tornata, M. turbinata and M. littoralis close together. The average linkage dendrogram produced from the flavonoid data has M. littoralis as the closest Medicago species to M. noeana which is placed in Section Rotatae by Lesins and Lesins(1979) and Heyn(1963) while the minimum spanning tree has it closest to M. soleirolii. M. noeana shows the phytoalexin response of Medicago species(Ingham and Harborne 1976) and because of its closeness to the two Section Pachyspirae members M. littoralis and M. soleirolii, then this strongly suggests that M. noeana also belongs in Section Pachyspirae. Small(1981) placed M. noeana in Section Pachyspirae based on numerical analysis of 75 morphological characters. It should be noted however that M. soleirolii is not placed in Section Pachyspirae by all taxonomists. Heyn(1963) places it in Section Rotatae while Lesins and Lesins(1979) place it in Section Pachyspirae. The flavonoid chemistry tends to support the Lesins and Lesins(1979) classification of this species. Cytological evidence of Simon and Simon(1965) also suggested that M. soleirolii belongs in Section Pachyspirae. The two species M. murex and M. constricta are considered to be members of

the Section Pachyspirae but the flavonoid data of the present study suggests that these species do not belong here. The serological data of Simon (1979) and an analysis of floral characteristics (Small et al 1981b) also suggested that M. murex does not belong with the Section Pachyspirae. Once again, both the serological data and the flavonoid data complement each other and indicate that M. murex is closely related to M. coronata (Section Leptospirae) and M. granadensis (Section Intertextae).

Traditionally, M. granadensis, M. intertexta and M. muricoleptis form the Section Intertextae; however, the serological data and flavonoid data agree that M. intertexta and M. granadensis are very different from each other.

The Section Rotatae traditionally consists of the species, M. scutellata, M. rotata, M. rugosa and M. blancheana with Lesins and Lesins (1979) and Heyn (1963) also including M. noeana. In the minimum spanning tree, only M. rugosa and M. blancheana appear to be closely related. Actually, one member of Section Rotatae, M. rotata provides a central link between members of the Section Leptospirae except for the species M. praecox, M. minima and M. arabica. Serological data of Simon (1979) also suggest that M. praecox does not belong with the Section Leptospirae and that M. arabica is a "peculiar" species.

Heyn(1963) considers the Subgenus Spirocarpos to be morphologically advanced, evolving through the Section Falcago of the Subgenus Medicago. Cross pollination by insects prevails in the Section Falcago whereas Subgenus Spirocarpos is exclusively self-pollinated. A tripping mechanism occurs in both. Heyn(1963) cites this as strong evidence that Subgenus Spirocarpos evolved from Section Falcago since the proper functioning of the tripping mechanism is essential for reproduction in the latter but it is a relic in the former, self-pollinating group.

There are thought to be two trends in flavonoid evolution 1. a reduction in structural complexity and in number of flavonoid structures, 2. diversification in the flavonoid nucleus involving extra oxygenation, O-methylation etc.(Gornall and Bohm 1978). These trends lead to difficulties in interpreting flavonoid patterns because the presence of simple flavonoid structures could represent either a primitive state or a secondary reduction and therefore an advanced state.

Within the Leguminosae there are woody and herbaceous genera. The presence of proanthocyanidins is correlated with woodiness, a supposedly primitive character and therefore species possessing them are considered to be primitive. The genus Acacia is woody and possesses myricetin, quercetin and kaempferol but no flavones(Harborne 1971). The genus

Genista is herbaceous and possesses quercetin, kaempferol as well as luteolin and other flavones but no myricetin (Harborne 1971). The genus Medicago shows the latter pattern of flavonoids and lacks proanthocyanidins in the leaves and so may be considered an advanced genus within the Leguminosae.

It is difficult to assess chemical evolutionary trends in the genus on the basis of the few compounds that have been tentatively identified. However, the perennials of Medicago show a tendency toward lacking flavonols and possessing flavones. This would suggest an advanced state especially since tricin is prominent in this Subgenus and this compound has two O-methyl groups on the B-ring. This is contrary to the evolution that Heyn (1963) suggests based on morphology.

To be useful as taxonomic characters, flavonoids should be correlated with other characters (Harborne 1975). A comparison of the degree of agreement with each other of several subsets of morphological characters of the 47 Medicago species examined and with the flavonoid data of the present study, reveal that the flavonoid chemistry is not quite as consistent with the traditional classification of Medicago as are vegetative morphology, fruit morphology and flower morphology. This is to be expected since it is mostly these characters that have been used to give the traditional structure to the genus. On the whole, the flavonoid chemis-

try shows strong agreement with the traditional classification of Medicago especially with Subgenus Medicago, Subgenus Orbicularia and Subgenus Spirocarpos Section Pachyspirae.

The flavonoid chemistry also supports some suggested affinities of controversial species(refer to Table 1). M. arborea belongs in Subgenus Medicago, M. soleirolii and M. noeana belong in Subgenus Spirocarpos Section Pachyspirae, the problematical species M. platycarpa, M. ruthenica and M. rubescens belong in the Medicago genus, the divergent species M. carstiensis, M. radiata, M. orbicularis and M. heyniana belong in Subgenus Orbicularia along with M. platycarpa and M. ruthenica, M. hybrida is a link between Subgenus Medicago and Subgenus Orbicularia.

The flavonoid chemistry suggests that M. lupulina and M. secundiflora are not as close as the morphology indicates, that M. murex does not belong in Subgenus Spirocarpos Section Pachyspirae, that M. murex is close to M. coronata and M. granadensis, that M. granadensis and M. intertexta are very different, that M. praecox does not belong with Subgenus Spirocarpos Section Leptospirae and that M. arabica is a "peculiar" species. All the above results are supported by similar results from other sources such as phytoalexin, serological, pollen and floral studies.

The flavonoid chemistry and numerical analysis are strong tools in the study of the taxonomy of the genus Medicago.

The various numerical techniques did give slightly different results but generally the strongly defined groupings are maintained and only the weakly defined relationships tend to be unstable.

The identification of more of the aglycones present in the genus Medicago as well as the flavonoids of the two closely related genera Trigonella and Melilotus may allow better separation of the genera and species. Full identification of the compounds would also reveal if any phytoalexins are present. The production of phytoalexins would be a reflection of the physiological state of the plant. and in this way would distort the taxonomy so produced unless all species under study could be induced to produce them. Only then would they be taxonomically significant. Individual compounds to be studied more closely as possible markers of Medicago species include apigenin and compounds #23 and #24. Also studies involving glycosides would probably provide additional information which would be useful in taxonomically structuring the genus.

BIBLIOGRAPHY

Adler and Trainer (1960). Vet. Rec. 72:1171-72.

Alston, R. E., T.J. Mabry and B. L. Turner (1963). Perspectives in Chemotaxonomy;Studies of secondary compounds in plants may provide knowledge of phytogenetic relationships. Science 142 (3592):545-552.

---------- and B. L. Turner (1963). Biochemical Systematics, Prentice Hall Inc., London.

Anonymous, (1971). Census of Canada, Agriculture. Statistics Canada Volume 4.1 page 5.

Anonymous, (1973). Protein Sources for Livestock. Agriculture Canada Publication #1515.

Armstrong, Nowosad, Ripley and Kalbfleisch (1948). Canada Department of Agriculture Publication 735, 34p.

Barnes, D. K. (1966). Flower color inheritance in diploid and tetraploid alfalfa: a reevaluation. USDA Tech. Bull. 1353.

Bate-Smith, E. C. (1954). Biochem. J. 58:122.

---------- and N. H. Lerner (1954). Leucoanthocyanins II, Systematic distribution of leucoanthocyanins in leaves. Biochem J. 58:126-132.

---------- and P. Ribereau-Gayon (1959). Qualitas Plantarium et Material Vegetabiles 8:189-98.

Bickoff, E. M., A. L. Livingston and S. C. Witt (1965a). Identification of 7,4'-dihydroxyflavone in forages. Phytochem. 4:523-24.

----------, ----------, ----------, R. E. Lundin and R. R. Spencer (1965b). Isolation of 4'-O-methylcoumestrol from alfalfa. J. Agric. Fd. Chem 13:597-599.

----------, R. R. Spencer, S. C. Witt and B. E. Knuckes (1969). Studies on the Chemical and Biological Properties of Coumestrol and Related Compounds. USDA Tech. Bull. 1408.

----------, S. C. Witt and A. L. Livingston (1965c). 3',4',7-trihydroxyflavone in alfalfa. J. Pharm. Sci. 54:1555-1556.

Boissier, E. (1849). Diagones plantarum orientalium novarum. Ser. I, No. 9. Paris.

Bolton, J. L. (1962). Alfalfa, World Crop Series, Leonard Hill Interscience.

----------, B. P. Goplen and H. Baenziger (1972). in Alfalfa Science and Technology, C. Hanson, ed., American Society of Agronomy Inc. Madison, Wisconsin, U.S.A.

Briggs, D. and S. M. Walters (1969). Plant Variation and Evolution. McGraw-Hill, New York and Toronto.

Buttery, B. R. and R. I. Buzzell (1973). Varietal differences in leaf flavonoids of soybean. Crop Sci 13:103-106.

Clifford, H. T. and W. Stephenson (1975). An Introduction to Numerical Classification. Academic Press, London and New York.

Collins, F. W. C. (1979). personal communication.

Cooper, R. L. and F. C. Elliot (1964). Flower pigments in diploid alfalfa. Crop Sci 4:367-371.

Dass, H. C. and G. M. Weaver (1972). Cellulose thin-layer chromatography of phenolic substances. J. Chrcmat. 67:105-111.

Dewick, P. M. and M. Martin (1976). Biosynthesis of Isoflavonoid Phytoalexins in Medicago sativa; the biosynthetic Relationship between Pterocarpans and 2'-hydroxyisoflavans. J. Chem. Soc. Chem. Comm. 637-638.

Egger, K. and M. Keil (1965). Separation of mixtures of flavones by partition chromatography on polyamide. Z. Anal. Chem. 210:201-208.

Ferguson, W. S., D. B. Ashworth and P. A. Terry (1950). Nature 166:117.

Freudenberg, K. and K. Weinges (1960). Notation and Nomenclature of Flavonoids. Tetrahedron 8:336-49.

Gibbs, R. D. (1974). Chemotaxonomy of Flowering Plants, McGill-Queens University Press.

Gillett, J. and D. White (1978). Checklist of Vascular Plants of the Ottawa-Hull Region, Canada. National Museums of Canada.

Gleason, H. A. and A. Cronquist (1963). Manual of Vascular Plants. D. Van Nostrand Co., New York.

Gornall, R. J. and B. A. Bohm (1978). Angiosperm Flavonoid Evolution: A reappraisal. Systematic Bot. 3(4):353-368.

Gower, J. C. (1966). A Q-technique for the calculation of canonical variates. Biometrika 53:588-590.

---------- (1971). Statistical methods of comparing multivariate analyses of the same data. Proc. first conf. math. archaeol. hist. sci:138-149.

Guggolz, J., A. L. Livingston and E. M. Bickoff (1961). Forage Estrogens: Detection of Daidzein, Formononetin, Genistein, and Biochanin A in Forages. J. Agr. and Fd. Chem. 9:330-332.

Hanson, C. H. (1972). in Alfalfa Science and Technology, C.H. Hanson ed. American Society of Agronomy Inc. Madison, Wisconsin, U.S.A.

---------- and Davis (1972). ibid.

Harborne, J. B. (1965). Plant Polyphenols XIV, Characterization of flavonoid glycosides by acidic and enzymatic hydrolyses. Phytochem 4:107-120.

---------- (1967). Comparative Biochemistry of the Flavonoids, Academic Press, London and New York.

---------- (1969). Chemosystematics of the Leguminosae, Flavonoid and Isoflavonoid patterns in the Tribe Genisteae. Phytochem 8:1449-1456.

---------- (1971). Chemotaxonomy of the Leguminosae, J. B. Harborne, D. Boulter and B. L. Turner eds. Chapter 2, Academic Press, London and New York.

---------- (1975) in The Flavonoids. J. B. Harborne, T. J. Mabry and H. Mabry eds. Chapman and Hall, London.

---------- (1979). personal comunication.

Hendrick, U.P. (1919). Sturtevant's Notes on Edible Plants, J. B. Lyon, Albany, New York 686p.

Heyn, C. C. (1963). The Annual Species of Medicago. Scripta Hierosolomytana. Vol 12 (Hebrew Univ:Jerusalem) Magnes Press.

Ingham, J. L. (1977). Medicarpin as a Phytoalexin of the Genus Melilotus. Z. Naturforsch 32C:449-452.

---------- (1979). Isoflavonoid Phytoalexins of the Genus Medicago. Biochemical Systematics and Ecology 7:29-34.

---------- and J. B. Harborne (1976). Phytoalexin Induction as a new dynamic approach to the study of systematic relationships among higher plants. Nature 260:241-243.

---------- and B. L. Millar (1973). Sativin: an Induced Isoflavan from the leaves of Medicago sativa L. Nature:125-126.

Krishnamurty, H. G. (1980). personal communication.

Lesins, K. A. and I. Lesins (1979). Genus Medicago(Leguminosae), A taxonomic study. W. Junk, The Hague.

Livingston, A. L., S. C. Witt, F. E. Lundin and E. M. Bickoff (1965). Medicagol, a new coumestan from alfalfa. J. Org. Chem. 30:2353-2355.

Mabry, T. J., K. R. Markham and M. B. Thomas (1970). The Systematic Identification of Flavonoids, Springer-Verlag, Berlin.

McCollum, E. V., E. Orent-Keiles and H. G. Day (1939). The Newer Knowledge of Nutrition, MacMillan Co., New York.

Monties, B., M. Chesneaux and C. H. Hutin (1976). Caracterisation des cultivars a l'aide de différence quantitatives de teneur en composés polyphencliques: Application au Mais et a L'oeillet., Ann. Amélior. Plantes 26(4):495-516.

Newby, V. K., R. Sablon, R. Synge, K. V. Casteele and C. G. Van Sumere (1980). Free and bound phenolic acids of Lucerne(Medicago sativa cv Europe). Phytochem 19:651-657.

Niemann, G. J. (1979). Improved amine spray reagent for the detection of sugars. J. Chromat. 170:227.

Robinson, T. (1975). The Organic Constituents of Higher Plants, 3rd Edition, Cordess Press.

Seikel, M. (1964). in Biochemistry of phenolic compounds, J. B. Harborne ed. p33-76, Academic Press, London and New York.

Seringe, N. C. (1825). Medicago, Trigonella, Pocockia in Prodromus systematis naturalis regni vegetabilis, Vol. 2 edited by A. P. de Candolle, Paris 171-185.

Simon, J. P. (1967). Relationship in Annual Species of Medicago V. Analysis of phenolics by means of one-dimensional chromatographic techniques. Aust. J. Bot 15:83-93.

---------- (1979). Serological Studies in Medicago, Melilotus and Trigonella (Leguminosae) II. Relationships of critical taxa of Sections Medicago and Lupularia of the Genus Medicago. Bot. Gaz. 140(4):452-460.

---------- and D. W. Goodall (1968). Relationship in Annual Species of Medicago VI. Two-dimensional chromatography of the phenolics and analysis of the results by probabilistic similarity methods. Aust. J. Bot. 16:89-100.

---------- and A. Simon (1965). Relationship in Annual Species of Medicago I. Number and morphology of chromosomes. Aust. J. Agric. Res. 16:37-50.

Singh, K. and B. D. Thompson (1961). The possibility of identification of vegetable varieties by paper chromatography of flavonoid compounds. Proc. Amer. Soc. Hortic. Sci. 77: 520-527.

Small, E. (1981). A numerical analysis of major groupings in Medicago employing traditional characters. Can. J. Bot. (in press).

---------, I. J. Bassett and C. W. Crompton (1981a). Pollen variation in Tribe Trigonelleae (Leguminosae) with special reference to Medicago. Pollen et Spores (submitted).

---------, C. W. Crompton and B. S. Brookes (1981b). The taxonomic value of floral characters in tribe Trigonellae (Leguminosae), with special reference to Medicago. Can. J. Bot. (in press).

Smith, D. G., A. G. McInnes, V. J. Higgins and P. L. Millar (1971). Nature of the phytoalexin produced by alfalfa in response to fungal infection. Physicl. Pl. Pathcl. 1:41-44.

Smith, I. (1960). Chromatographic and electrophoretic techniques. Vol 1:291-308 Heineman Med. Book, London.

Sneath, P. H. A. and R. R. Sokal (1973). Numerical Taxonomy. Freeman, San Francisco.

Sokal, R. R. and P. H. A. Sneath (1966). Efficiency in Taxonomy. Taxon 15:1-21.

Spencer, R. R., E. M. Bickoff, R. E. Lundin and B. E. Knuckes (1966a). Lucernol and sativol, two new coumestans from alfalfa (Medicago sativa). J. Agric. Fd. Chem.. 14:162-165.

---------- B. E. Knuckes and E. M. Bickoff (1966b). 7-hydroxy-11,12-dimethoxycoumestan, Characterization and synthesis. J. Org. Chem. 31:988-989.

Stathem, C. M. and A. K. Crowden (1974). Anthocyanin biosynthesis in Pisum sequence studies of pigment production. Phytochem. 13:1835-1840.

Thakur, M. (1980). A study of Anthocyanin Pigments of Impatiens and their bearing on the taxonomy of this genus. M. Sc. Thesis, University of Ottawa, Canada.

Turner, B. L. and R. E. Alston (1959). Segreation and recombination of chemical constituents in a hybrid swarm of Baptisa laevicaulis X Baptisa viridis and their taxonomic implications. Am. J. Botan. 46:678-86.

Vassilczenko, I. T. (1972). Genus novum Rhodusia Vass. E familia papilionacearum. Novitates systematicae plantarum vascularium, Acad. Sc. URSS, Inst. Bot. V. L. Komarovii, 9:203-206 (in Russian).

Vierma de Bezada, M. (1980). Effect of Environmental Conditions on Lentil Seeds. M.Sc. Thesis, University of Ottawa, Canada.

Appendix A

TABLE 8

Seed Sources

Population	Growth Conditions	Source	Origin
M. arabica 625	F	8	France
888	F	13	
M. arborea 103	G	14	
220	G	24 P.I.249937	Greece
M. blancheana 353	F	25	USSR
459	F	8	Hungary
M. cancellata 150	F	23 P.I.315458	USSR
1024	G	6 UAG#43	
M. carstiensis 68	F and G	2	
M. constrictsa 343	F	6 UAG#1272	
M. coronata 12	F	20	
1019	F	20	
M. cretacea 56	G	22	
M. daghestanica 1023	G	6 UAG#67	
M. disciformis 1016	F	20	
M. doliata 333	F	6 UAG#1974	
368	F	25	Morrocco
M. granadensis H2984	G		
M. heyniana 342	F	6 UAG#2303	
M. hybrida 57	F	22	
350	F	25	
M. intertexta 568	F	8	Hungary
965	F	4	
M. laciniata 594	F	8	Denmark
M. lanigera H2984	G		
M. littoralis 100	F	10	
955	F	24	Tunisia
M. lupulina 311	F	3	
318	F	9	
871	F	16	
M. marina 110	G	12	
120	G	18	
M. minima 332	G	15	
381	G	5	
M. murex 1021	F	20	Greece
M. muricoleptis 347	G	6 UAG#768	
M. noeana 338	G	6 UAG#832	
M. orbicularis 316	F	11	unknown
400	F	1	
M. pironae 159	F	23 P.I.253449	Yugoslavia
672	F	23 P.I.253449	Yugoslavia
M. platycarpa 161	G	23 P.I.258759	USSR
164	G	23 P.I.260994	USSR
M. polymorpha 360	F	25	Kreta

M. praecox 337 434	F	8		France
M. prostrata 699 700	F	6	UAG#957	
	F	8		Hungary
M. pubescens 957	F	8		France
M. radiata T177	F	24	P.I.245002	India
M. rigidula 711 986	F	8		Holland
	F	8		Jaffa
M. rotata 335 723	F	17		
	F	6	UAG#280	
M. rugosa 39 1018	F	8		Portugal
	F	20		
M. ruthenica 1025	G	20		Crete
		6	UAG#539	

M. sativa S223	F	21		
M. sauvagei 334	F	6	UAG#539	
M. saxatilis H586				
M. scutellata 899	F	14		
1020	G	20		Greece
M. secundiflora 339	G	6	UAG#553	
M. soleirolii 336	F	6	UAG#567	
M. suffruticosa 199	G	23	P.I.299054	Minnesota
750	F	6		France
M. tenoreana 341	G	6	UAG#1499	
M. tornata 929	F	24	P.I.384644	Morrocco
970	F	19		
M. truncatula 939	F	24	P.I.283662	Italy
943	F	24	P.I.292436	Israel
M. turbinata 366	F	25		CSSR
945	F	24	P.I.197387	Australia

Growth conditions; G=greenhouse, F=field

Sources
1. Botanischer Garten der Universitat, Frankfurt.
2. Botanischer Garten der Universitat, Wein, Austria.
3. Botanischer Garten, Oldenberg.
4. Commonwealth Sci. and Ind. Res., Canberra, Australia.
5. Conservatoire et Jardin botaniques de Ville de Genève, Genève.
6. Dr. K. Lesins, Department of Genetics, University of Alberta, Edmonton.
7. Dr. T. Neubauer, Botanischer Garten, DDR.
8. Forage Crops, C.E.F., Ottawa.
9. Hortus Botanicus Centralis Academiae Scientiarum, BSSR.
10. Hortus Botanicus Universitatus Portudensis, Portugal.
11. Hortus Botanicus Universitatis Varsaviensis, Warsaw; Poland.
12. Instituto di Botanica, Della Universita, Pisa, Italy.
13. Jardin Botanique, Angers, France.
14. Jardin Botanique de Bordeaux, France.
15. Jardin Experimental Jean Massart, Universite de Bruxelles, Belgique.
16. Martin-Luther-Universitat, Halle-Wittenberg, DDR.
17. Montpellier, France.
18. Museum National D'Histoire Naturelle, Services des Cultures, Paris, France.
19. Rabat, Morrocco.
20. Royal Botanic Garden, Kew, England.
21. Station for Agrobotany, Tapioszele, Hungary.
22. Station Nationale d'Essais de Semences, Service Botanique, Versailles, France.
23. USDA, Ames, Iowa.
24. USDA, Georgia
25. Zentralinstutut fur Genetik und Kulturpflanzenforschung der Akademie der Wissenschaften der DDR, Gatersleben.

TABLE 9

Results for All Populations Studied

	Compound Number																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
SATI S223	0	0	0	1	1	1	1	1	0	1	0	0	1	1	1	1	1	1	1	1	1	0	1	1
PROS 699	0	0	1	1	1	1	1	1	0	1	0	0	0	1	1	0	1	1	1	1	1	0	1	1
PROS 700	0	0	0	1	1	1	1	1	0	1	0	0	1	1	1	1	1	0	0	0	0	0	1	1
CANC 150	0	0	0	0	1	1	1	1	0	1	1	0	0	0	0	0	0	1	1	1	0	0	1	1
CANC 1024	0	0	0	1	1	1	1	1	0	1	1	1	1	0	1	1	0	1	1	1	1	0	1	1
SAXA H586	0	0	1	0	1	1	1	1	0	1	0	0	1	0	1	0	1	1	0	0	0	0	1	1
DAGH 1023	0	0	1	0	1	1	1	1	0	1	0	0	1	1	1	1	1	1	1	0	0	0	1	1
PIRO 159	0	0	0	1	1	1	1	1	0	1	0	0	1	1	1	1	1	1	0	0	0	0	1	1
PIRO 672	0	0	0	1	1	1	1	1	0	1	0	0	1	1	1	1	1	1	1	0	0	0	1	1
MARI 110	0	0	0	0	1	1	1	1	0	1	0	0	0	0	0	0	1	1	0	0	0	0	1	1
MARI 120	0	0	0	0	1	1	1	1	0	1	0	0	0	0	0	0	0	1	1	1	1	0	1	1
ARBO 103	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	1	1	1	0	0	0	1	1
ARBO 220	1	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	1	1	1	1	1	0	1	1
HYBR 57	0	1	0	1	0	1	1	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	1
HYBR 350	1	1	0	1	0	1	1	0	0	0	0	1	1	1	1	1	1	0	1	1	1	0	1	1
SUFF 199	1	0	1	0	1	1	1	0	0	0	0	1	0	1	1	0	0	0	0	1	1	0	1	1
SUFF 750	1	1	1	1	0	1	1	0	0	0	0	1	0	1	1	0	0	0	1	1	1	0	1	1
STCU 339	0	0	1	0	0	1	1	1	0	0	0	1	0	0	0	0	1	1	1	1	1	0	1	1
LUPU 311	1	1	1	1	0	1	1	0	1	1	1	0	1	1	1	0	1	0	1	1	1	0	1	1
LUPU 318	0	1	1	0	0	1	1	0	1	0	1	0	0	1	0	0	1	0	1	1	1	0	1	1
LUPU 871	1	1	1	1	0	1	1	0	0	1	1	0	1	1	1	0	1	0	1	1	1	0	1	1
CPET 56	1	0	1	0	1	0	0	0	1	1	1	0	0	1	0	0	0	0	1	0	0	0	1	1
CARS 68	0	0	1	0	0	1	0	0	1	0	1	0	0	1	1	0	0	0	1	1	1	0	1	1
CARS 466	1	1	0	1	0	1	0	0	1	1	1	0	1	1	1	0	1	0	1	1	1	0	1	1
FADI T177	1	1	1	1	0	1	1	0	1	1	1	0	1	1	1	0	1	0	0	1	1	0	1	1
ORBI 316	1	0	1	1	0	1	0	0	1	1	1	0	1	1	1	0	1	0	1	1	1	0	1	1
ORBI 400	1	0	1	1	0	1	0	0	1	1	1	0	1	1	1	0	0	0	1	1	1	0	1	1
HEYN 342	1	0	1	1	1	1	0	0	1	1	1	0	1	1	1	0	0	0	1	1	1	0	1	1
PUBE 957	1	1	0	1	0	1	1	1	0	1	1	0	1	0	1	0	0	0	1	1	1	0	1	1
PLAT 161	1	1	1	1	0	1	1	0	1	0	1	0	1	1	1	0	1	0	0	0	0	0	1	1
PLAT 164	1	1	0	1	0	1	1	0	1	1	0	0	1	1	1	0	0	0	0	0	0	0	1	1
RUTH 1025	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	0	1	0	0	0	1	1

1 OF 1

TABLE 9

Results for All Populations Studied

	Compound Number																										
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
23	0	0	0	1	1	1	1	1	0	1	0	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
9	0	0	1	1	1	1	1	1	0	1	0	0	0	1	1	0	1	1	1	1	1	0	1	1	1	1	0	0
0	0	0	0	1	1	1	1	1	0	1	0	0	1	1	1	1	1	0	0	0	0	0	1	1	1	1	0	0
0	0	0	0	0	1	1	1	1	0	1	1	0	0	0	0	0	0	1	1	1	0	0	1	1	0	0	1	0
24	0	0	0	1	1	1	1	1	0	1	1	1	1	0	1	1	0	1	1	1	1	0	1	1	0	0	1	0
86	0	0	1	0	1	1	1	1	0	1	0	0	1	0	1	0	1	1	0	0	0	0	1	1	0	1	0	0
23	0	0	1	0	1	1	1	1	0	1	0	0	1	1	1	1	1	1	1	0	0	0	1	1	0	1	1	0
9	0	0	0	1	1	1	1	1	0	1	0	0	1	1	1	1	1	1	0	0	0	0	1	1	1	1	1	0
2	0	0	0	1	1	1	1	1	0	1	0	0	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1	0
0	0	0	0	0	1	1	1	1	0	1	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0
0	0	0	0	0	1	1	1	1	0	1	0	0	0	0	0	0	0	1	1	1	1	0	1	1	0	0	0	0
3	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	1	1	1	0	0	0	1	1	1	1	1	0
0	1	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	1	1	1	1	1	0	1	1	1	1	1	0
	0	1	0	1	0	1	1	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	1	1	1	1	0
0	1	1	0	1	0	1	1	0	0	0	0	0	1	1	1	1	1	1	0	1	1	1	0	1	1	0	1	1	0
9	1	0	1	0	1	1	1	0	0	0	0	0	1	0	1	1	0	0	0	0	1	1	0	1	1	0	1	1	0
0	1	1	1	1	0	1	1	0	0	0	0	0	1	0	1	1	0	0	0	1	1	1	0	1	1	0	1	1	0
9	0	0	1	0	0	1	1	1	0	0	0	0	1	0	0	0	0	1	1	1	1	1	0	1	1	1	1	1	0
1	1	1	1	1	0	1	1	0	1	1	1	1	0	1	1	1	0	1	0	1	1	1	0	1	1	1	1	0	0
8	0	1	1	0	0	1	1	0	1	0	1	0	0	1	0	0	1	0	1	1	1	0	1	1	1	1	0	0
1	1	1	1	1	0	1	1	0	0	1	1	0	1	1	1	0	1	0	1	1	1	0	1	1	0	1	0	0
	1	0	1	0	1	0	0	0	1	1	1	0	0	1	0	0	0	0	1	0	0	0	1	1	0	0	0	0
	0	0	1	0	0	1	0	0	1	0	1	0	0	1	0	0	0	0	1	1	1	0	1	1	1	1	1	0
6	1	1	0	1	0	1	0	0	1	1	1	0	1	1	1	0	1	0	1	1	1	0	1	1	1	1	1	0
77	1	1	1	1	0	1	1	0	1	1	1	0	1	1	1	0	1	0	0	1	1	0	1	1	0	1	1	0
6	1	0	1	1	0	1	0	0	1	1	1	0	1	1	1	0	1	0	1	1	1	0	1	1	1	1	1	0
0	1	0	1	1	0	1	0	0	1	1	1	0	1	1	1	0	0	0	1	1	1	0	1	1	0	1	1	0
2	1	0	1	1	1	1	0	0	1	1	1	0	1	1	1	0	0	0	1	1	1	0	1	1	0	0	1	0
7	1	1	0	1	0	1	1	1	0	1	1	0	1	0	1	0	0	0	1	1	1	0	1	1	1	1	1	0
1	1	1	1	1	0	1	1	0	1	0	1	0	1	1	1	0	1	0	0	0	0	0	1	1	0	1	1	0
4	1	1	0	1	0	1	1	0	1	1	0	0	1	1	1	0	0	0	0	0	0	0	1	1	0	1	1	0
25	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	0	1	0	0	0	1	1	0	1	1	0

continued

	Compound Number																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	2
MINI 332	0	1	0	1	0	1	0	0	0	1	0	0	1	0	0	0	1	0	1	0	0	1	1	
MINI 381	0	1	0	1	0	1	1	0	1	0	0	0	1	0	0	0	1	0	0	0	0	1	1	
LANI H2984	1	0	0	0	1	1	1	1	1	1	1	0	0	1	1	0	1	0	1	1	1	0	1	
TENO 341	1	0	0	1	1	1	0	0	1	1	1	0	0	1	0	0	0	0	1	1	1	1	1	
SAUV 334	1	0	1	1	0	1	0	0	1	1	1	0	1	1	1	0	1	0	1	1	1	0	1	
DISC 1016	0	0	0	1	0	1	0	0	0	1	1	0	1	1	0	0	1	0	1	0	0	0	1	
LACI 594	1	1	1	1	1	1	0	0	1	1	1	0	1	1	1	0	0	0	1	1	1	0	1	
CORO 12	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	0	1	
CORO 1019	1	1	0	1	1	1	1	1	1	1	1	0	0	0	1	0	0	0	1	1	1	0	1	
PRAE 337	0	0	0	1	1	1	1	1	0	1	0	0	0	1	1	1	1	0	1	1	1	0	1	
POLY 360	0	1	0	1	1	1	1	0	0	1	0	0	1	1	1	0	1	0	1	0	0	0	1	
POLY 434	1	1	0	1	1	1	1	0	0	1	1	0	1	1	1	0	1	0	1	1	0	0	1	
APAB 625	1	0	0	1	0	1	1	1	0	1	0	0	0	1	1	0	0	0	1	1	1	0	1	
APAB 888	1	0	0	1	0	1	1	1	0	1	0	1	0	1	1	0	0	0	1	1	1	0	1	
NOEA 338	0	0	1	1	1	1	1	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	
CONS 343	0	1	0	0	0	1	1	0	0	1	0	0	1	1	0	0	1	0	1	0	0	0	1	
PIGI 711	0	1	0	1	1	1	1	1	0	1	0	0	0	0	1	0	1	1	1	1	1	0	1	
PIGI 986	0	1	0	0	1	1	1	1	0	1	0	0	1	1	1	0	1	1	1	1	1	0	1	
LITT 100	1	0	0	0	1	1	1	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	
LITT 955	1	0	0	0	1	1	1	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	
TORN 929	1	0	1	1	1	1	1	1	1	1	0	0	0	1	1	0	0	1	0	0	0	0	1	1
TORN 970	0	0	1	0	1	1	1	1	1	1	0	0	0	0	1	0	1	1	1	0	0	0	1	1
TRUN 939	0	0	1	1	1	1	1	1	1	0	0	0	0	1	1	0	1	1	1	0	0	0	1	1
TRUN 943	0	0	1	1	1	1	1	1	1	0	0	0	0	1	1	0	1	1	0	0	0	0	1	1
SOLE 336	0	0	1	0	1	1	1	1	1	0	0	0	0	0	1	0	0	1	0	1	0	0	1	1
TURB 366	0	0	1	1	1	1	1	1	1	1	0	0	1	1	1	0	1	1	0	0	0	0	1	1
TURB 945	0	0	1	1	1	1	1	1	1	1	0	0	1	0	1	0	1	1	0	0	0	0	1	1
MURE 1021	1	1	0	1	0	1	1	0	1	1	1	0	1	1	1	0	0	0	1	1	1	0	1	1
DOLI 333	0	1	0	1	1	1	1	1	1	1	0	0	1	0	1	0	1	0	1	1	1	0	1	1
DOLI 368	0	1	0	1	1	1	1	1	1	1	0	0	1	1	1	0	1	0	0	1	1	0	1	1
GRAN H2984	1	0	1	1	0	1	1	0	1	1	1	0	1	1	1	0	0	0	0	0	0	0	1	1
MURI 347	0	0	1	0	0	1	1	0	0	1	0	0	0	0	1	0	1	0	1	1	1	0	1	1
INTE 568	1	1	0	1	0	1	1	0	1	1	1	1	1	1	1	0	1	0	1	1	1	0	1	1
INTE 965	1	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	0	1	1
ROTA 335	1	0	1	1	1	1	1	0	1	1	1	0	0	1	1	0	1	0	1	1	1	1	1	1
ROTA 723	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	0	1	0	1	1	1	1	1	1
BLAN 353	1	1	1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	1	1
BLAN 459	1	1	1	1	0	1	1	0	0	1	0	1	0	1	0	1	0	0	0	0	1	0	0	0
RUGO 39	1	0	1	1	0	1	1	0	0	1	0	0	1	1	1	0	0	0	1	1	1	0	1	1
RUGO 1018	1	0	1	1	0	1	1	0	0	1	0	1	1	1	1	0	0	0	1	1	1	0	1	1
SCUT 899	0	1	0	1	0	1	1	0	0	1	0	0	1	0	1	0	0	0	1	0	0	0	1	1
SCUT 1020	0	0	1	1	0	1	1	0	0	1	0	0	0	0	1	0	0	0	0	1	1	0	1	1

A list of full species names is given in Appendix Table 10.

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	Compound Number																											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
332	0	1	0	1	0	1	0	0	0	1	0	0	1	0	0	0	1	0	1	0	0	1	1	1	0	1	0	0
881	0	1	0	1	0	1	1	0	1	0	0	0	1	0	0	0	1	0	0	0	0	1	1	1	0	1	0	0
12984	1	0	0	0	1	1	1	1	1	1	1	0	0	1	1	0	1	0	1	1	1	0	1	1	0	1	0	0
841	1	0	0	1	1	1	0	0	1	1	1	0	0	1	0	0	0	0	1	1	1	1	1	1	1	1	1	0
834	1	0	1	1	0	1	0	0	1	1	1	0	1	1	1	0	1	0	1	1	1	0	1	1	1	1	0	0
016	0	0	0	1	0	1	0	0	0	1	1	0	1	1	0	0	1	0	1	0	0	0	1	1	1	1	1	0
694	1	1	1	1	1	1	0	0	1	1	1	0	1	1	1	0	0	0	1	1	1	0	1	1	1	1	1	0
72	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	0	1	1	1	1	1	0
019	1	1	0	1	1	1	1	1	1	1	1	0	0	0	1	0	0	0	1	1	1	0	1	1	0	1	1	0
137	0	0	0	1	1	1	1	1	0	1	0	0	0	1	1	1	1	0	1	1	1	0	1	1	1	1	1	0
660	0	1	0	1	1	1	1	0	0	1	0	0	1	1	1	0	1	0	1	0	0	0	1	1	1	1	1	0
734	1	1	0	1	1	1	1	0	0	1	1	0	1	1	1	0	1	0	1	1	0	0	1	1	1	1	1	0
525	1	0	0	1	0	1	1	1	0	1	0	0	0	1	1	0	0	0	1	1	1	0	1	1	1	1	1	0
888	1	0	0	1	0	1	1	1	0	1	0	1	0	1	1	0	0	0	1	1	1	0	1	1	1	1	1	0
538	0	0	1	1	1	1	1	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	1	0	1	0	0
643	0	1	0	0	0	1	1	0	0	1	0	0	1	1	0	0	1	0	1	0	0	0	1	1	0	1	0	0
711	0	1	0	1	1	1	1	1	0	1	0	0	0	0	1	0	1	1	1	1	1	0	1	1	1	1	1	0
986	0	1	0	0	1	1	1	1	0	1	0	0	1	1	1	0	1	1	1	1	1	0	1	1	0	1	1	0
100	1	0	0	0	1	1	1	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	1	1	0	1	0
955	1	0	0	0	1	1	1	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	1	0
929	1	0	1	1	1	1	1	1	1	1	0	0	0	1	1	0	0	1	0	0	0	0	1	1	1	1	1	0
970	0	0	1	0	1	1	1	1	1	1	0	0	0	0	1	0	1	1	1	0	0	0	1	1	1	1	1	0
939	0	0	1	1	1	1	1	1	1	0	0	0	0	1	1	0	1	1	1	0	0	0	1	1	1	1	1	0
943	0	0	1	1	1	1	1	1	1	0	0	0	0	1	1	0	1	1	0	0	0	0	1	1	1	1	1	0
136	0	0	1	0	1	1	1	1	1	0	0	0	0	0	1	0	0	1	0	1	0	0	1	1	0	1	1	0
866	0	0	1	1	1	1	1	1	1	1	0	0	1	1	1	0	1	1	0	0	0	0	1	1	1	1	1	0
945	0	0	1	1	1	1	1	1	1	1	0	0	1	0	1	0	1	1	0	0	0	0	1	1	1	1	1	0
1021	1	1	0	1	0	1	1	0	1	1	1	0	1	1	1	0	0	0	1	1	1	0	1	1	0	1	1	0
133	0	1	0	1	1	1	1	1	1	1	0	0	1	0	1	0	1	0	1	1	1	0	1	1	1	1	0	0
168	0	1	0	1	1	1	1	1	1	1	0	0	1	1	1	0	1	0	0	1	1	0	1	1	1	1	1	0
12984	1	0	1	1	0	1	1	0	1	1	1	0	1	1	1	0	0	0	0	0	0	0	1	1	0	0	1	0
147	0	0	1	0	0	1	1	0	0	1	0	0	0	0	1	0	1	0	1	1	1	0	1	1	0	1	0	0
568	1	1	0	1	0	1	1	0	1	1	1	1	1	1	1	0	1	0	1	1	1	0	1	1	1	0	0	0
965	1	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	0	1	1	0	0	0	0
135	1	0	1	1	1	1	1	0	1	1	1	0	0	1	1	0	1	0	1	1	1	1	1	1	1	1	1	0
723	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	0
153	1	1	1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	1	1	0	1	1	0
159	1	1	1	1	0	1	1	0	0	1	0	1	0	1	0	1	0	0	0	0	1	0	0	0	1	1	0	0
9	1	0	1	1	0	1	1	0	0	1	0	0	1	1	1	0	0	0	1	1	1	0	1	1	1	1	1	0
018	1	0	1	1	0	1	1	0	0	1	0	1	1	1	1	0	0	0	1	1	1	0	1	1	1	1	1	0
899	0	1	0	1	0	1	1	0	0	1	0	0	1	0	1	0	0	0	1	0	0	0	1	1	1	1	1	0
020	0	0	1	1	0	1	1	0	0	1	0	0	0	0	1	0	0	0	0	1	1	0	1	1	0	1	1	0

of full species names is given in Appendix Table 10.

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TABLE 10

Abbreviations Used for Species

ARAB=Medicago arabica
ARBO=M. arborea
BLAN=M. blancheana
CANC=M. cancellata
CARS=M. carstiensis
CONS=M. constricta
CORO=M. coronata
CRET=M. cretacea
DAGH=M. daghestanica
DISC=M. disciformis
DOLI=M. doliata
GRAN=M. granadensis
HEYN=M. heyniana
HYBR=M. hybrida
INTE=M. intertexta
LACI=M. laciniata
LANI=M. lanigera
LITT=M. littoralis
LUPU=M. lupulina
MARI=M. marina
MINI=M. minima
MURE=M. murex
MURI=M. muricoleptis
NOEA=M. noeana
ORBI=M. orbicularis
PIRO=M. pironae
PLAT=M. platycarpa
POLY=M. polymorpha
PRAE=M. praecox
PROS=M. prostrata
PUBE=M. pubescens
RADI=M. radiata
RIGI=M. rigidula
ROTA=M. rotata
RUGO=M. rugosa
RUTH=M. ruthenica
SATI=M. sativa
SAUV=M. sauvagei
SAXA=M. saxatilis
SCUT=M. scutellata
SECU=M. secundiflora
SOLE=M. soleirolii
SUFF=M. suffruticosa
TENO=M. tenoreana
TORN=M. tornata
TRUN=M. truncatula
TURB=M. turbinata
MELI=Melilotus alba
TCAE=Trigonella caerulea
TCOR=Trigonella corniculata

Authorities may be found in Small(1981).