A BIOSYSTEMATIC STUDY OF THE *Carduus nutans* L. COMPLEX IN CANADA

presented by
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July, 1987

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of a Master of Science (M.Sc.)

ABSTRACT

The Carduus nutans L. complex in North America has been treated either as one species with four subspecies (ssp. nutans, ssp. leiophyllus (Petrovic) Stoj. & Stef., ssp. macrolepis (Peter.) Kazmi, and ssp. macrocephalus (Desf.) Nyman) or as three species: Carduus nutans with two subspecies (ssp. nutans and ssp. macrolepis), C. thoermeri Weinm, and C. macrocephalus Desf.. A biosystematic study of this complex, including morphological, flavonoid and isoenzyme analyses, of 19 populations, was conducted in order to clarify the taxonomy of this complex in Canada. Both the morphological and flavonoid analyses clearly indicate the existence of only two closely related groups of taxa referable to ssp. nutans and ssp. leiophyllus. The classificatory discriminant analysis showed that in these two taxa only 6% of the individuals were misclassified. Each taxon is characterized by a flavonoid profile. Given the high genetic identity value ($\bar{I}$=0.93) between the two taxa in the complex, and the estimates of genetic variability obtained, the taxa are best treated at the subspecific level, as ssp. nutans and ssp. leiophyllus.

RESUME

Le complexe du Carduus nutans en Amérique du Nord a été traité soit comme quatre sous-espèces (ssp. nutans, ssp. leiophyllus (Petrovic) Stoj. & Stef., ssp. macrolepis (Peter.) Kazmi, et ssp. macrocephalus (Desf.) Nyman) ou comme trois espèces: C. nutans avec deux sous-espèces (ssp. nutans et ssp. leiophyllus), C. thoermeri Weinm., et C. macrocephalus Desf.. Une étude de la biosystematique de ce complexe au Canada a été entreprise dans le but d'en clarifier la taxonomie. Cette étude comporte des analyses sur la constitution en flavonoides ainsi que des analyses morphologiques et électrophorétiques portant sur 19 populations. Les analyses sur la constitution en flavonoides et les analyses morphologiques indiquent la présence de deux proches taxons au Canada. L'analyse discriminante indique que seulement 6% des individus sont mal classés. Chaque taxon est caracterizé par un profile des flavonoides. Les analyses électrophorétiques quant à elles ne permettent pas de distinguer les deux taxons révelés par les deux autres types d'analyses; la fréquence allélique des enzymes étudiés ne variant pas suffisemment d'un taxon a l'autre. La variation obtenue dans les analyses, suggère que les deux taxons reconnus dans le complexe du C. nutans, soient traités au niveau sous-specifique c'est-à-dire commme ssp. nutans et ssp. leiophyllus.

ACKNOWLEDGEMENT

I wish to express my sincere thanks to Dr. J.F. Bain whose advice and continuous assistance during the course of this study went beyond all expectations. I thank him for his patient guidance and constructive comments provided during the preparation of this manuscript. His skill as a supervisor, and his financial support facilitated my time and work at Macdonald College, and made its conclusions possible.

I would also like to thank Dr. S.I. Warwick for her valuable help during this study, especially in the application of electrophoretic and statistical techniques, the use of her laboratory for the isozyme analysis, and for her helpful comments pertaining to the preparation of this manuscript.

I wish to express my gratitude to the rest of my committee, especially to Ms M.J. Waterway, whose sound advice and interest shown in my work was greatly appreciated.

Thank you to Dr. W.F. Grant and J. Raelson for their constructive advice pertaining to the application of cytological techniques, and to Mr. J. Woods from the Ministry of Agriculture and Food for the collection of seeds sent from British Columbia.

I would also like to extend my appreciation to my fellow students, especially to Jeannette Whitton and Julie Desrosiers for their help and friendship.

Finally, a special thank to Dr. D.L. Smith who was able to put a smile on my face even during the more difficult moments.

FOREWORD

This thesis is submitted under the form of a single manuscript according to the conditions outlined in the Guidelines concerning thesis preparation, which are as follows:

"The Candidate has the option, subject to the approval of the Department, of including as part of the thesis the text of an original paper, or papers, suitable for submission to learned journals for publication. In this case the thesis must still conform to all other requirements explained in Guidelines Concerning Thesis Preparation. Additional material (experimental and design data as well as descriptions of equipment) must be provided in sufficient detail to allow a clear and precise judgement to be made of the importance and originality of the research reported. Abstract, full introduction and conclusion must be included, and where more than one manuscript appears, connecting texts and common abstracts, introduction and conclusions are required. A mere collection of manuscripts in not acceptable; nor can reprints of published papers be accepted.

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Although all the work was under the responsability of the candidate, the project was supervised by Dr. J.F. Bain from Macdonald College of McGill University and Dr. S.I. Warwick from Agriculture Canada (Biosystematic Research Institute, Ottawa). Consequently, both are co-author of the manuscript submitted. This manuscript will be submitted to the Canadian Journal of Botany.

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INTRODUCTION

The genus Carduus is native to the Old World where its indigenous distribution extends over Europe, central Asia and East Africa. The first revision of the entire genus was done by Kazmi (1963,1964), who recognized 91 species, 59 hybrids and several infraspecific taxa. Franco (1976) described 48 species of Carduus in Flora Europaea. Representatives of the genus cover numerous geographical and/or ecological areas and several taxa have become weeds. Some species have been introduced in several areas of the world such as Australia, South Africa, and North and South America (Kazmi, 1964).

Members of Carduus have been referred to as the plumeless thistles and can easily be distinguished from Cirsium, the 'true' or plumed thistle, by their not having feathery pappus but rather filamentous hairs attached to the seeds. Basic chromosome numbers of 8, 9, and 11 have been reported (Kazmi, 1964).

The species of Carduus were first introduced to the east coast of North America in the late 1800's (Dunn, 1976; Moore and Frankton, 1974; Mulligan and Frankton, 1954; Stuckey and Forsyth, 1971). As many as eight taxa have been described in North America. McCarty (1978) recognized seven species and separated them into three groups: the slender-flowered thistle group (C. tenuiflorus Curt. and C. pycnocephalus L.), the small-flowered group (C. acanthoides L. and C. crispus L.), and the large-flowered group (C. nutans

L., C. thoermeri Weinm., and C. macrocephalus Desf.). Only three of these species are recognized in Canada: C. nutans, C. acanthoides, and C. crispus (Moore and Frankton, 1974; Mulligan and Frankton, 1954).

Taxonomic problems in North America arise only in the treatment of the large-flowered Carduus group. In Kazmi's (1964) monograph, this group is referred to as the series Nutantes Kazmi, and it includes seven species. Franco (1976) described eight species in this group. Of these species, three (C. nutans, C. thoermeri, and C. macrocephalus) are recognized by Boldt (1978) and by McCarty (1978) to be present in North America. In their treatment of Carduus in Canada, Mulligan and Frankton (1954), and Moore and Frankton (1974) only recognized the existence of C. nutans in the nutans group. However, Moore and Frankton (1974) included three subspecies under C. nutans: ssp. nutans, ssp. leiophyllus (Petrovic) Stoj. & Stef., and ssp. macrolepis (Peterm.) Kazmi. C. thoermeri is listed by the latter authors as a synonym of C. nutans ssp. leiophyllus. McGregor (1986) partly disagrees with the above treatments. In the region covered by the Flora of the Great Plains, he recognized ssp. leiophyllus and included ssp. macrolepis in this taxon. He also recognized C. macrocephalus but at the subspecific level. In North America, C. macrocephalus has only been described from the United States. In spite of the differences between these treatments the authors all used the same morphological characters to

separate the taxa. Presence of pubescence on the plant distinguishes ssp. nutans and macrocephalus from the other two taxa and ssp. nutans can be separated from ssp. macrocephalus based on its smaller head diameter and phyllaries. The two other taxa, ssp. leiophyllus (or C. thoermeri) and ssp. macrolepis are both glabrous to slightly pubescent and Moore and Frankton (1974) report that ssp. macrolepis can be separated from ssp. leiophyllus based on its larger head and the shape of the phyllaries.

Clearly, the North American treatments are not in agreement. The main differences are: 1) the taxonomic status of ssp. leiophyllus and its relationship to C. thoermeri, 2) the recognition of ssp. macrolepis in Canada , and 3) the presence of ssp. macrocephalus in Canada.

In the Old World there also exist contrasting classifications of the nutans group. The major treatments that were examined for this study are the following: Arènes' (1949) treatment which reviewed the taxonomy of five species of Carduus (C. nutans, C. acanthoides, C. crispus, C. defloratus L., and C. nigrescens Vill.); Kazmi's (1963, 1964) monograph on the genus Carduus; and Franco's (1976) treatment of the European members of Carduus. Only the taxa of concern for the North American treatments of the nutans group will be reviewed here. Arènes (1949) treated the taxa in the nutans group all as subspecies or varieties of C. nutans (Table 1). By contrast, in this group Kazmi (1964) and Franco (1976)

TABLE 1 - Comparison of the Old World treatments for the nutans group.

Arènes (1949)	Kazmi (1964)	Franco (1976)
C. nutans ssp. eu-nutans Gugl.	C. nutans L. ssp. nutans	C. nutans ssp. nutans
C. nutans ssp. eu-nutans Gugl. var. typicus Fiori subvar. roseus J. Ar.		
C. nutans ssp. eu-nutans Gugl. var. typicus Fiori f. albiflorus Estival		
C. nutans var. typicus Fiori subvar. simplex (Coss. et Germ.) Chass. et J. Ar.		
C. nutans ssp. eu-nutans Gugl. var. typicus Chass. et J. Ar. f. albiflorus (Esti.) Chass. et J. Ar.		
C. nutans ssp. eu-nutans Gugl. var. typicus Chass. et J. Ar. f. roseus (J. Ar.) Chass. et J. Ar.		
C. nutans var alpina Gren.	C. nutans ssp. macrolepis (Peterm.) Kazmi	Does not mention this taxon
C. nutans ssp. eu-nutans Gugl. var. macrolepis (Peterm.) J. Ar.		
C. nutans ssp. leiophyllus (Petr.) J. Ar.	C. thoermeri Weinm.	C. thoermeri Weinm.
C. nutans ssp. leiophyllus (Petr.) J. Ar. var. petrovicii J. Ar.		
C. nutans ssp. leiophyllus (Petr.) J. Ar. var. heldreichii J. Ar.		
C. nutans ssp. leiophyllus (Petr.) J. Ar, var. stribrnyi J. Ar.		
C. nutans ssp. eu-nutans Gugl. var. kirghisensis J. Ar.		

recognized three species: C. nutans, C. macrocephalus, and C. thoermeri. Kazmi (1964) included two subspecies under C. nutans (ssp. nutans and ssp. macrolepis), while Franco did not mention ssp. macrolepis in his treatment. The morphological variation among the taxa is continuous and both Arènes and Franco agree that the nutans group in the Old World includes taxa that are difficult to recognize and that only a few extreme taxa can be easily identified. Thus in both the New and Old World the nutans group is a poorly understood complex.

Several biological aspects of the group have been documented which serve to further demonstrate the group's variability. In most of these studies the taxa were referred to as C. nutans s.l. or as C. thoermeri s.l.. Studies which investigated the life cycle patterns of musk thistle (Lee and Hamrick, 1983; McCarty and Scifres, 1969; McCarty et al., 1969;) have all reported that musk thistle acts as a biennial, winter annual, or as an annual. The flowering period of the plant differs according to its geographical location. In Kansas and in Nebraska, flowering may start as early as May and June, and may continue for as long as eight weeks depending on the weather conditions (Lee and Hamrick, 1983; McCarty et al. 1984). In Canada, Harris (1984) observed that in Saskatchewan C. nutans had a short and intense flowering period which finished by the end of July. Pollination in musk thistle is accomplished by several insect species, predominantly Bombus spp., Apis spp., and Lepidoptera (Smyth

and Hamrick, 1984). It is primarily an outcrossing species but is also self-fertile, particularly in northern populations (McCarty et al., 1980; Smyth and Hamrick, 1984; Warwick, 1987).

A number of studies dealt with seed production, germination and dispersal in this species (Hamrick and Lee, 1987; McCarty, 1964; McCarty 1982; McCarty and Scifres, 1969; Smith and Kok, 1984). Individual plants were found to average nearly 10,000 achenes per plant (McCarty, 1964) and when quality of achenes and percent of germination were taken into account, potentially more than 3,000 seedlings per plant could be produced (McCarty and Scifres, 1969). The achenes are mainly dispersed by wind. Based on results of a laboratory experiment Smith and Kok (1984) reported that most seeds were deposited within 50 m of the point of release. McCarty et al. (1969) reported no innate seed dormancy and that given adequate moisture and warmth, seeds can germinate soon after dispersal.

Hamrick and Lee (1987) and Lee and Hamrick (1983) studied the demography of natural populations of C. nutans in eastern Kansas, and the physical environmental mechanisms that influence levels of germination, survival and growth in a greenhouse experiment. Both studies concluded that germination can occur over an extended period of time and that optimum levels of germination, survival and growth occur in moist environments where infraspecific competition is low.

Austin et al. (1985) also suggested that C. nutans tends to be a poor competitor in a multispecies community. Lee and Hamrick (1983) found that increased plant density leads to smaller rosette sizes which in turn negatively affects seed production. Variation in demography of musk thistle was thought to be the result of phenotypic plasticity rather than genetic differences.

Although much is known on certain aspects of the biology of C. nutans s.l. very little of this information has been integrated with the taxonomic treatments of this complex in North America. This paper presents the results obtained from a biosystematic study of the C. nutans complex in Canada and in two northern states of the United States. The main purposes of this study were: 1) to determine whether ssp. leiophyllus should be treated at the subspecific level or at the specific level (as C. thoermeri), and 2) to determine whether ssp. macrolepis should be recognized in Canada. For this purpose morphological, flavonoid, and electrophoretic analyses were undertaken.

MATERIALS AND METHODS

The eighteen populations of C. nutans used for all analyses were collected across Canada and in the northern part of the United States during the summers of 1985 and 1986. The locations are listed in Table 2. Flower heads and seed heads were collected from 30 different mother plants in the field, and when possible the flower and seed heads were collected from the same individual. Leaf material was collected in bulk for each population. Voucher specimens were also collected from each population and are deposited at the McGill University Herbarium (MTMG). An additional population, from British Columbia, was used only in the flavonoid and isozyme analyses since only seed material was available at the time of collection. All of the above collections were population samples and will be referred to as such for the remainder of the text so that they may be distinguished from herbarium specimens. Herbarium specimens were obtained from ACAD, ALTA, CAN, DAO, DAS, GFND, GH, KANU, MIN, MONTU, NFLD, QFA, QK, QUE, RMS, SASK, SFS, TRTE, UAC, UBC, UNB, US, USAS, WIN (Holmgren and Keuken, 1974). Some of these specimens were used for morphological measurements in order to permit a comparison to be made between the population samples and the previous taxonomic treatments. Details follow in the morphology section.

TABLE 2 - Collection sites of the populations of *Carduus nutans* used in the study.

*231 - Chatham, Northumberland Co., N.B., roadside (65°05'N 47°00'W)
*232 - Rexton, Kent Co., N.B., pasture (64°08'N 46°07'W)
*243 - Glen Morris, Brant Co., Ont., pasture (43°16'N 80°21'W)
*245 - Kirkwall, Wentworth Co. Ont., pasture (43°21'N 80°10'W)
*246 - Acton, Halton Co., Ont., pasture (43°37'N 80°02'W)
248 - Durham (E of) Grey Co., Ont., pasture (44°10'N 80°49'W)
249 - Durham (NE of), Grey Co., Ont., pasture (44°10'N 80°49'W)
250 - Havelock, Peterborough Co., Ont., pasture (44°26'N 77°53'W)
*252 - Calgary, Alta, open field along the river (51°03'N 114°05'W)
256 - Findlater, Sask., waste field (50°47'N 105°24'W)
260 - Regina, Sask., along Ring Road (50°27'N 104°37'W)
261 - Kaleida, Man., pasture (49°08'N 98°28'W)
267 - Morden, Man., pasture (49°11'N 98°06'W)
269 - Walhalla, N.Dak., pasture (48°08'N 97°09'W)
273 - Gardar, N.Dak., waste field (48°06'N 97°08'W)
280 - Ellıston, Mont., open field (46°05'N 112° 04'W)
285 - Ravallı, Mont., pasture (47°03'N 114°01'W)
297 - Montreal, Qué., waste field (45°30'N 73°33'W)
BC - Princeton, B.C. (49°28'N 120°30'W)

* Populations for which collection time necessitated that most flower and seed heads be collected from different mother plants

Morphology

Twenty-two morphological characters were measured for 18 individuals per population; 15 quantitative and seven qualitative characters (Table 3). To measure the quantitative characters, flower heads were soaked in warm water for approximately five minutes. For all populations the flower heads used for morphological measurements were approximately at the same stage of maturity i.e. pollen was present in the anthers of the flowers both in the centre and outer edge of the head. Some of the characters listed in Table 3 require more explicit definitions. They are as follows:

Head diameter 1 (HDD1): measured from population samples only. The flower heads were cut in half longitudinally and the widest portion of the head including the outermost phyllaries was measured.

Head diameter 2 (HDD2): measured on herbarium material only. Maximum head width was measured. This usually occurs across the upper portion of the corollas.

Phyllary length - from the basal row (PLO): length of the phyllary, from its point of attachment to the end of the spine.

TABLE 3 - Characters used in the morphological analysis.

<u>Quantitative characters</u>	
Head diameter 1	HDD1
Head diameter 2	**HDD2
Phyllary length, basal row	PLO
Phyllary width, basal row	PWO
Phyllary length, middle row: above constriction	PLA
below constriction	PLB
Phyllary width, middle row: above constriction	*PWMT
below constriction	PWMB
Spine length of phyllary, from middle row	SPLT
First leaf distance from the base of the flower head	HDD
The same flower was used for: corolla tube length	COR1
pappus length	PAP
anther length	ANT
Achene length	SDLT
Achene width	SDWI
Achene weight (for 10 achenes)	SDWE
<u>Qualitative characters</u>	
Phyllary midrib (1-prominent; 2-not prominent)	MID
Phyllary reflexion (5 angle grade scales)	REF
Phyllary constriction (1-present; 2-absent)	*CONS
Phyllary pubescence (present/absent)	*PHP
Leaf pubescence (present/absent)	**STP
Phyllary apex (1-acute; 2-acuminate)	*PHAP
Phyllary shape - above constriction (1-lanceolate; 2-ovate)	PHSH1
Phyllary shape - below constriction (1-oval; 2-linear)	PHSH2

* Characters also recorded on herbarium specimens
**Characters recorded only on herbarium specimens

Phyllary width - from the basal row (PWO):
maximum width of phyllary. (There is no constriction on the basal phyllary.)

The following four measurements were made on the same phyllary, taken from the third or fourth row from the outside.

Phyllary length - above constriction - (from the middle row) (PLA):
length from the constriction to the apex of the phyllary including the spine.

Phyllary length - below constriction - (from the middle row) (PLB):
length between the constriction and the point of attachment of the phyllary.

Phyllary width - above constriction - (from the middle row) (PWMT):
maximum width of the portion of phyllary above the constriction.

Phyllary width - below constriction - (from the middle row) (PWMB):
minimum width of the portion of phyllary below the constriction.

Phyllary reflexion (REF): angle at which the upper portion of the phyllary deviates from the lower portion. 1. 180-225° (least reflexed) 2. 135-180° 3. 90-135° 4. 45-90° 5. 0-45° (most reflexed).

Phyllary pubescence (PHP): 1. glabrous to slightly pubescent 2. moderately to densely pubescent.

Leaf pubescence (STP): recorded on herbarium specimens only. All leaves on the plants were examined. 1. glabrous to slightly pubescent 2. moderately to densely pubescent.

Many of the seven qualitative characters included in this study have also been used to separate the taxa in previous taxonomic treatments. They were recorded to allow easy comparison between the results of this and previous studies. Some of these characters, notably phyllary pubescence, were also used for defining a priori groups to be used and compared in the canonical and discriminant analyses. None of the qualitative characters were used in the canonical or discriminant analyses.

Six characters were measured on 76 herbarium specimens collected in Canada, representing the specimens annotated or identified by any of the following authors: R.J. Moore, C. Frankton, G.A. Mulligan, and R.L. McGregor. These characters,

listed in Table 3, were measured to verify if the results obtained by these authors were comparable with those of this study. The measurements recorded on herbarium specimens were not included in the statistical analyses described below.

Statistical Analysis

All statistical analyses were performed on the main-frame computer facilities of McGill University System of Interactive Computing (MUSIC system) using the subroutines ANOVA, CANDISC, and DISCRIM available in SAS (SAS Institute 1982, 1985). One-way analyses of variance were performed on each of the 15 morphological characters. T-tests were carried out on each of the character means obtained for the two groups defined *a priori*. In order to explore the possible relationships among the populations, two canonical discriminant analyses (CANDISC) were carried out: 1) using the 18 population samples as statistical groups, and 2) using two groups as suggested by the results obtained from the analysis of the qualitative characters. In both cases the same 15 quantitative characters listed in Table 3 were included in the analyses. Discriminant analysis (DISCRIM) was carried out to estimate the correct classification rates of the individuals assigned to each of the two groups used in the second canonical analysis. The 99% confidence limits of the canonical means of populations samples (analysis 1), shown in

Figs. 1 and 2 as circles around the mean, were calculated with a radius of $t_{(0.01)}/N_1^{1/2}$ with N-1 degrees of freedom; N_1 is the number of individuals in group 1. The relative contribution of the characters to the canonical axes plotted in the figures is shown by a set of character vectors. Only the ten characters that contributed the most as determined by the length of the vectors were plotted. The positions of the end point of the vectors were obtained from the canonical loadings for each character on the respective axis. These were standardized by dividing each by the square root of the corresponding element of the diagonal of the inverse within-covariance matrix (Reyment et al., 1984).

Electrophoretic Analysis

Nineteen populations were assayed by horizontal starch gel electrophoresis for nine enzyme systems. From 13 to 48, 3-4 week old seedlings were surveyed for each population. Crude extracts were obtained by grinding one or two leaves in a small weighing boat containing cold extraction buffer (0.1 M tris-HCl pH 7.5, containing 10 mM KCl, 10 mM $MgCl_2$, 1 mM EDTA, 0.4% triton X-100, and 14 mM 2-mercaptoethanol) with insoluble PVPP (40,000 mW). The extracts were maintained on ice. Horizontal electrophoresis was conducted on 9.5% starch gels made with different combinations of gel and electrode buffers according to the particular enzymes assayed. A tris-citric system at pH 8.3 (Gottlieb, 1981a) was used for triose

phosphate isomerase (TPI), phosphoglucose isomerase (PGI), and glutamate-oxaloacetate-transaminase (GOT). A histidine-HCl system at pH 7.0 (Warwick and Black, 1986) was used for isocitrate dehydrogenase (IDH). A histidine system at pH 5.7 (Warwick and Gottlieb, 1985) was used for malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6-PGD), and malic enzyme (ME). A tris-citric system at pH 7.8 (Warwick et al., 1984) for which the gel buffer was modified (1.13g tris, 0.56g citric acid (anhydrous), 0.46 g EDTA, 1 L distilled water) was used for phosphoglucomutase (PGM) and glyceraldehyde-3-phosphate dehydrogenase (G-3PD). In these systems all enzymes migrated toward the anode. Gels were run until the bromphenol blue front had migrated about 10 cm from the origin.

Enzyme assay conditions were modified after Gottlieb et al. (1985) and Warwick and Gottlieb (1985). The assay conditions for each enzyme are given in Table 4.

A genetic analysis of the banding patterns was conducted by examining the segregation patterns in progeny resulting from selfing individuals heterozygous for different alleles (Warwick and Black, in prep.).

The locus with the most anodal position was designated 1, the next 2, and so forth. For each locus, the alleles specifying the allozymes were called a,b,c, the closer allele

TABLE 4 — Enzyme assay conditions used in the electrophoretic study of *Carduus nutans*.

(GOT)[1]	α-Ketoglutaric acid	73	mg
	L-aspartic acid	266.2	mg
	PVP-40	1	g
	EDTA	100	mg
	Na_2HPO_4	2.84	g
	Distilled H_2O	100	ml
(TPI)	0.1 M tris-HCl (8.0)	75	ml
	DHAP	10	mg
	EDTA	30	mg
	NAD	23	mg
	Na_2 arsenate	345	mg
	Gly-PDH	200	units
	MTT	0.75	ml
	PMS	pinch	
(PGI)	0.1 M tris-HCl (8.3)	75	ml
	Fructose-6-phosphate	35	mg
	NADP	4	mg
	G 6PDH (40 units/ml)	0.75	ml
	0.1 M $MgCl_2$	4	mg
	MTT	0.75	ml
	PMS	pinch	
(6-PGD)	0.1 M tris-HCl	75	ml
	6-phosphogluconate (basalt)	45	mg
	NADP	4	mg
	0.1 M $MgCl_2$	4	ml
	PMS	pinch	
(MDH)	0.1 M tris-HCl 9.1	75	ml
	Malic acid	150	mg
	NAD	30	mg
	MTT	0.75	ml
	PMS	pinch	
(ME)	0.1 M tris HCl (unadjusted)	75	ml
	DL-malic acid	315	mg
	NADP	15	mg
	Stir above, titrate with HCl to pH 7.2		
	0.1 M $MgCl_2$	4	ml
	MTT	0.75	ml
	PMS	pinch	

(IDH)	0.1 M tris-HCl	75	ml
	Isocitrate	150	mg
	NADP	8	mg
	0.1 M $MgCl_2$	4	mg
	MTT	0.75	ml
	PMS	pinch	
(PGM)	0.1 M tris-HCl	75	ml
	Glucose 1-phophate	57	mg
	NADP	4	mg
	Glucose 1,6 disphosphate (5mg/33ml/H_2O)	4	ml
	G 6 PDH (40units/ml)	0.75	ml
	0.1 M $MgCl_2$	4	ml
	MTT	0.75	ml
	PMS	pinch	
(G3-PD)	0.1 M tris-HCl	75	ml
	Fructose 1-6 diphosphate	130	mg
	Aldolase	50	ul
	NADP	8	mg
	Na_2 arsenate	60	mg
	EDTA	90	mg
	0.1 M $MgCl_2$	4	mg
	MTT	0.75	ml
	PMS	pinch	

1 See text for full name of the enzymes.

to the front being designated as a. Standard genetic identities were computed utilizing the methodology of Nei (1972).

Flavonoid Analysis

For the flavonoid analysis, methanolic extracts of bulk leaf material were used as the flavonoid source. Standard two-dimensional paper chromatography methods (butanol-acetic acid-water (4:1:5, upper phase) (BAW), 15% acetic acid) were used to separate the flavonoid compounds. Comparison of the compounds isolated by this method with the profiles obtained on polyamide plates in an aqueous solvent revealed that the same compounds were separating on thin layer. The 19 populations were surveyed using polyamide TLC plates and an aqueous solvent (water-butanol-acetone-dioxane (70:15:10:5)). After the survey, one population containing all the compounds was selected for a flavonoid analysis. In addition, to verify the existence of within population variation in the flavonoid profiles, 12 to 24 individuals per population were surveyed for each population in the same manner as described above (TLC in aqueous solvent). For this survey leaf material of individuals 12 to 15 weeks old, grown in the greenhouse from seed collected from different mother plants was used.

For the identification of individual flavonoids, the crude extract was partitioned using ethyl acetate and chloroform. The ethyl acetate fraction was then separated using column chromatography (Polyclar AT-GAF Corp.) and 80% MeOH as solvent. The isolated fractions were purified by streaking them on Whatman No 1 paper using 15% acetic acid as solvent. R_f values of pure compounds were determined on Whatman No 1 paper in four solvent systems (BAW, H_2O, 15% acetic acid, 80% phenol) with 10 µl of a 10^{-3}M solution of rutin (quercetin-3-O-rutinoside) run simultaneously as a standard on each sheet. Ultraviolet spectral analysis was carried out using standard techniques (Marbry et al., 1970).

Glycosides were hydrolyzed for 50-60 minutes in a 1:1 mixture of 1 N HCl:MeOH, in a 100°C water bath. After hydrolysis the solution was evaporated to dryness, redissolved in distilled H_2O, and partitioned in ethyl acetate. The sugars contained in the lower aqueous fraction were identified by co-chromatography beside 30 µl of a 0.005 M standard sugar mixture on cellulose (Polygram cel 300) plates. The plates were run in a pyridine-ethyl acetate-acetic acid-water (36:36:7:21) solvent, stained using aniline-diphenylamine phosphate (Sherma, 1972), and unknown sugars identified by their relative mobilities and by their colour after staining.

Chromosome Numbers

Chromosome counts were determined for one to three individuals per population. Root tips from greenhouse material were pretreated in 0.002 M 8-hydroxyquinoline for 16-24 hours at 4°C and hydrolyzed in 5 N HCl for 2 hours 30 minutes. The chromosomes were stained with Feulgen Reagent (Darlington and LaCour, 1976) for 40 minutes. Slides were prepared by squashing the root-tips in a 10:1 mixture of 45% acetic acid and glycerol (Schlarbaum and Tsuchiya, 1981). As expected, all individuals examined had a chromosome number of 2n = 16.

RESULTS

Morphology

The results obtained for the qualitative measurements are listed in Table 5. Two characters separate the populations into two groups, the presence of pubescence on the phyllaries (PHP), and the angle of reflexion of the phyllaries. The individuals in the first seven populations have pubescent phyllaries and the individuals in the first six populations have phyllaries that are generally less reflexed than those in the last 12 populations. Each of the remaining characters was relatively uniform throughout the populations.

Means, F-values, and t-values for the 15 quantitative morphological characters are listed for each population in Table 6. Except for one character (PLB) the F-values indicate among-population differences for each character ($p<0.001$) and the t-values indicate between-subspecies differences for 10 characters ($p<0.05$).

The results of the first canonical analysis are presented in Figs. 1 and 2. The canonical means for the populations are plotted against the first two axes (Fig. 1), and against the first and third axes (Fig. 2). The first, second and third canonical axes accounted for 54%, 16% and 10% of the variation respectively.

As can be seen in Figs 1 and 2, the distribution of the canonical means of the 18 population samples is relatively continuous. The populations on the left side

TABLE 5 - Results obtained for the qualitative characters recorded on the population samples and herbarium specimens of Carduus nutans. The results are presented as percentages.

GROUPS: Population samples (n=18)		MID 1*	REF 2	REF 3	REF 4	REF 5	CONS 1	PHP 2	PHSH1 1	PHAP 2	PHSH2 2	STP 2
A (ssp. nutans)	231	100		50	50		100	100	100	100	100	-
	232	100		50	50		100	100	100	100	78	-
	243	100		28	72		100	100	100	100	100	-
	245	100	17	22	61		100	100	100	100	100	-
	246	100	5	22	67	5	100	100	100	100	100	-
	248	100		28	72		94	94	100	100	100	-
	249	100			100		100	100	100	100	100	-
B (ssp. leiophyllus)	250	78			33	67	94	11	61	94	100	-
	252	100			72	28	89	0	100	100	94	-
	256	100			5	95	100	0	100	100	100	-
	260	100			33	67	100	11	100	100	100	-
	261	100			5	95	100	0	100	100	100	-
	267	100			50	50	100	0	100	100	100	-
	269	100			28	72	100	0	100	100	100	-
	273	100			17	83	100	0	100	100	100	-
	280	100			28	72	100	0	100	100	61	-
	285	100			50	50	100	0	100	100	100	-
	297	100			89	11	61	0	100	94	67	-
Herbarium specimens												
ssp. nutans (n=33-39)							97	97	100			97
ssp. leiophyllus (n=33-39)							95	3	100			0
ssp. macrolepis (n=4)							80	25	100			0

* Character states explained in Table 3.

TABLE 6 - Means, F-values and t-values of the 15 quantitative characters measured on *Carduus nutans*
(All measurements are in cm unless specified)

GROUPS	POP	Hdd	Plo	Pwmt	Pwmb	Pwo	Pla	Plb	Splt	Dfl	Corl	Pap	Ant	Sdlt	Sdwi	Sdwe (0.1mg)
A	231	2.49	1.96	0.41	0.28	0.24	1.87	0.80	0.38	1.73	1.17	1.69	0.69	3.87	1.36	45.53
(ssp. *nutans*)	232	1.60	1.34	0.26	0.19	0.18	1.26	0.68	0.34	1.53	1.17	1.71	0.71	3.74	1.34	49.50
	243	1.72	1.26	0.28	0.22	0.22	1.37	0.63	0.33	1.90	1.04	1.62	0.66	3.64	1.46	55.32
	245	1.65	1.12	0.29	0.25	0.22	1.09	0.84	0.28	1.77	0.99	1.53	0.64	3.23	1.34	45.46
	246	1.94	1.34	0.35	0.26	0.22	1.36	0.65	0.34	1.65	1.07	1.76	0.69	3.60	1.35	51.01
	248	1.88	1.20	0.34	0.26	0.19	1.17	0.67	0.28	2.48	1.21	1.81	0.75	3.71	1.36	49.70
	249	1.95	1.13	0.31	0.24	0.20	0.98	0.74	0.28	1.56	1.13	1.78	0.71	3.62	1.38	48.14
Means :		1.89	1.34	0.32	0.24	0.21	1.3	0.72	0.32	1.8	1.11	1.7	0.69	3.63	1.37	49.24
Standard errors:		0.03	0.03	0.006	0.004	0.003	0.03	0.04	0.006	0.1	0.01	0.02	0.005	0.03	0.02	0.89
B	250	2.08	1.08	0.40	0.31	0.19	1.28	0.72	0.31	2.76	1.21	1.89	0.73	3.98	1.51	55.72
(ssp. *leiophyllus*)	252	2.06	1.01	0.52	0.36	0.24	1.28	0.68	0.20	9.58	1.25	1.99	0.76	4.86	1.57	76.07
	256	2.67	1.16	0.54	0.34	0.23	1.38	0.82	0.24	6.07	1.27	2.01	0.74	4.17	1.57	66.21
	260	2.29	1.26	0.46	0.34	0.27	1.43	0.81	0.27	8.07	1.34	2.07	0.77	4.51	1.55	74.89
	261	2.48	1.26	0.46	0.28	0.22	1.60	0.77	0.30	4.37	1.33	2.03	0.83	3.98	1.36	46.62
	267	2.40	1.16	0.39	0.26	0.19	1.29	0.64	0.31	4.72	1.29	2.01	0.82	3.96	1.53	58.47
	269	2.23	1.09	0.40	0.29	0.17	1.24	0.64	0.29	5.93	1.33	1.99	0.79	4.02	1.44	58.37
	273	2.11	1.10	0.32	0.25	0.18	1.39	0.74	0.28	6.43	1.34	2.26	0.80	4.11	1.45	56.37
	280	2.90	1.75	0.58	0.36	0.26	1.84	0.71	0.36	3.84	1.21	1.84	0.79	4.11	1.37	41.33
	283	2.41	1.17	0.53	0.34	0.23	1.29	0.66	0.28	7.08	1.36	1.90	0.77	4.35	1.41	55.78
	297	1.86	0.91	0.33	0.24	0.16	1.01	0.62	0.25	2.40	1.21	1.85	0.74	4.00	1.51	67.06
Means :		2.32	1.18	0.45	0.31	0.21	1.37	0.71	0.28	5.61	1.29	1.99	0.78	4.19	1.48	59.72
Standard errors:		0.03	0.12	0.008	0.005	0.004	0.02	0.008	0.005	0.31	0.01	0.02	0.005	0.03	0.008	1.08
F-values for all populations:		25.76	21.82	30.14	19.65	10.25	15.51	1.36ns	8.13	12.24	10.06	12.84	13.09	40.87	9.54	15.08
T-values :		3.08*	1.48ns	3.92*	3.40*	0ns	0.61ns	0ns	1.72ns	3.22*	3.50*	3.94*	3.87*	4.83*	2.63**	2.27**

**, *, significant at the 0.05 and 0.01 levels, respectively

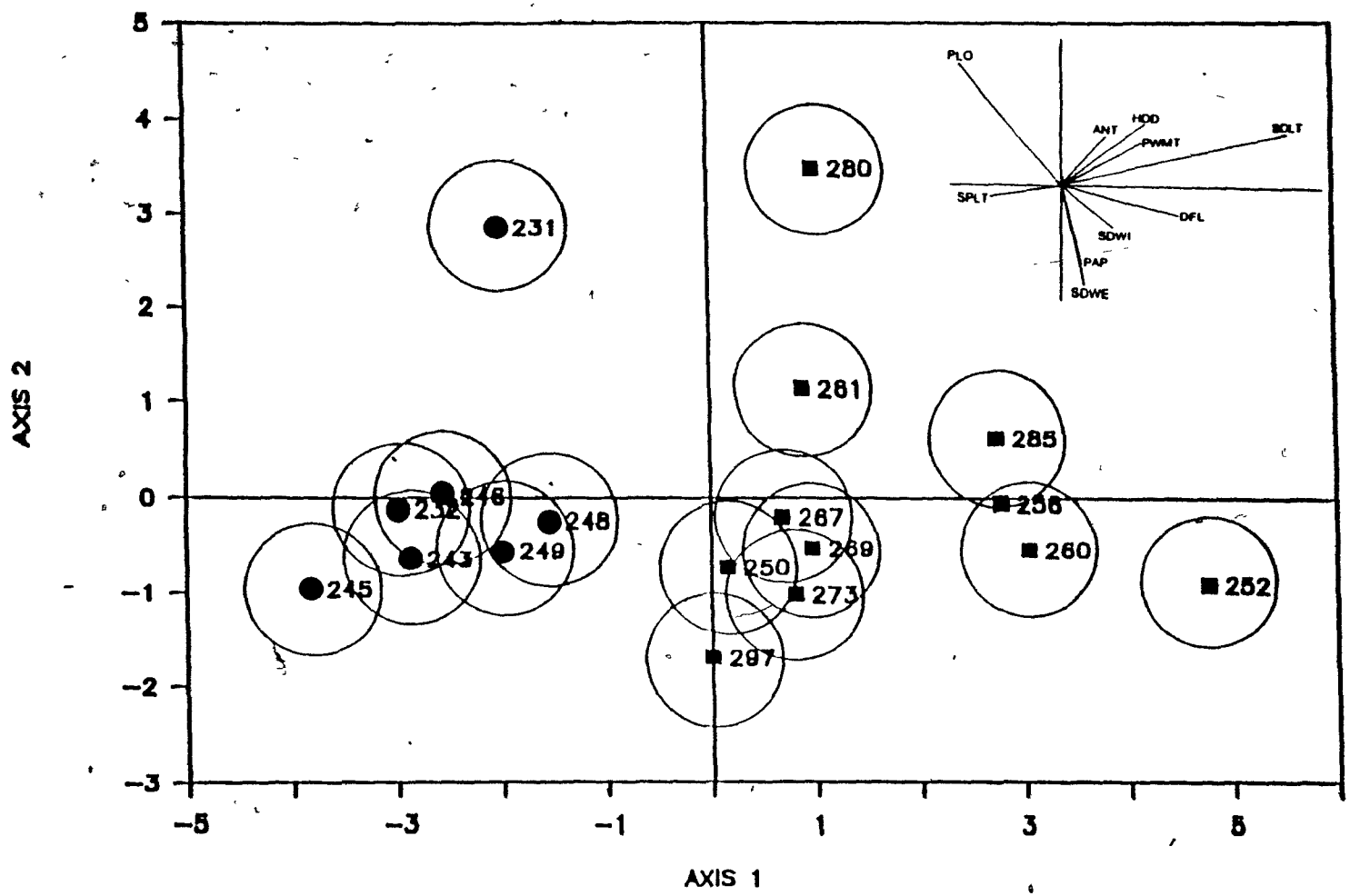

FIGURE 1 - Distribution of the canonical means against the first two axes for 18 population samples based on 15 morphological characters. Circles around each mean represent confidence limits at p=0.01. Character vectors showing the relative contribution of each character to the two axes are included.

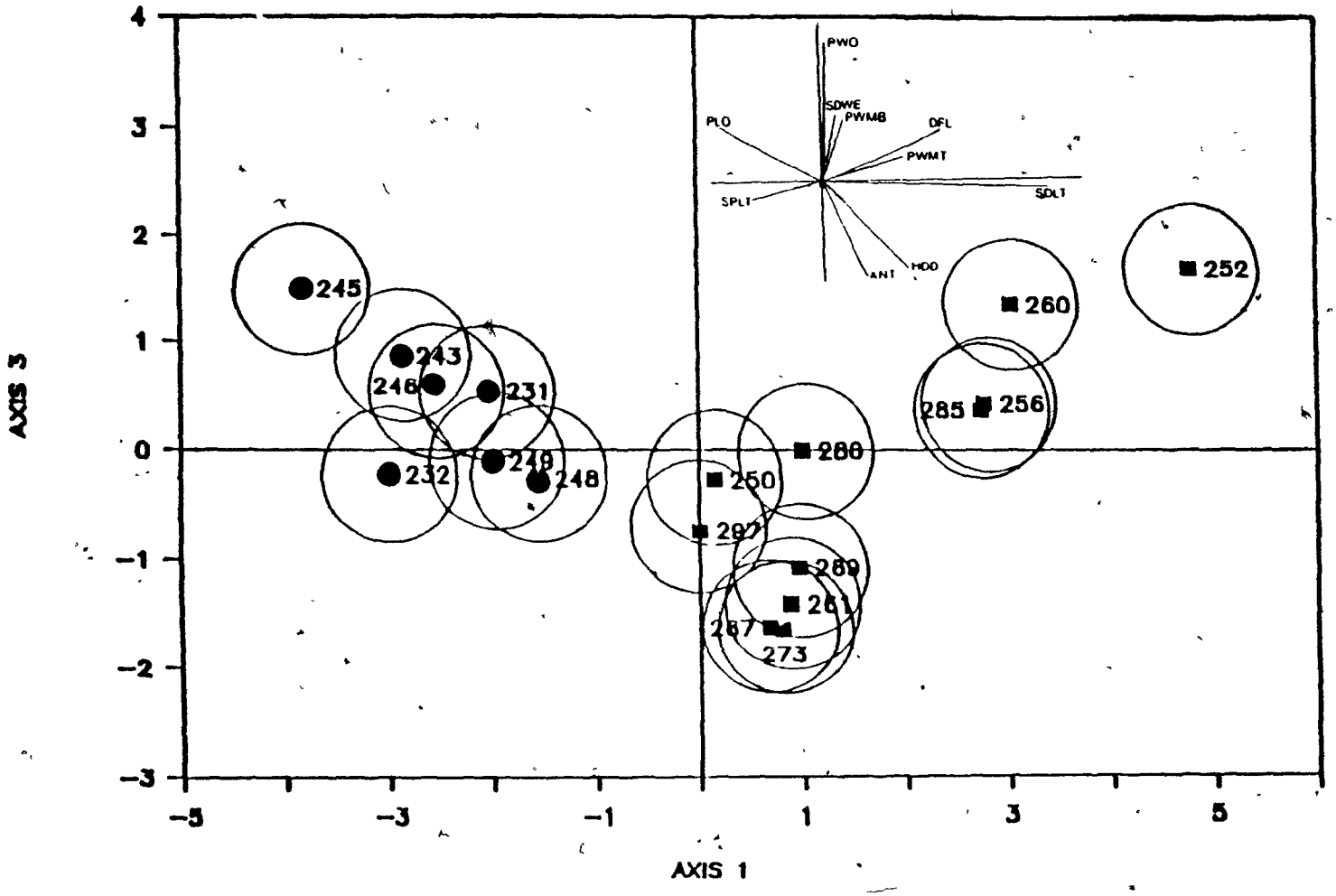

FIGURE 2- Distribution of the canonical means against the first and third axes for 18 population samples based on 15 morphological characters. Circles around each mean represent confidence limits at p=0.01. Character vectors showing the relative contribution of each character to the two axes are included.

of the graph were all collected in Ontario and New Brunswick, those on the right side include one Ontario (#250), and one Québec population (#297), while the rest were collected in Manitoba, Saskatchewan, Alberta, Montana, and North Dakota.

In Fig. 1 three populations, #231, #280, and #252, are distinctly separated from the other populations. Populations #231 and #280 contain individuals that are characterized by longer basal phyllaries, a longer upper portion of the middle row phyllary, and by larger head diameter. When the means of the populations are plotted against canonical axes 1 and 3, both populations clustered with the others (Fig. 2). For these two populations terminal flower heads or flower heads from the first axillary branches were collected. These are usually larger than the lower flower heads collected for all other populations and this difference may be responsible for the separation of #231 and #280 from the other populations. Population #252 was distinguished by its shorter spine length, longer achene length, and heavier achene weight.

A second canonical analysis was performed using only two groups. All individuals that had moderately to densely pubescent phyllaries were assigned to group A. All remaining individuals were included in group B. In the present study no other single character was useful for defining <u>a priori</u> groups (Table 6). Figure 3 presents the frequency distribution of canonical scores for the individuals in the two groups. These groups separated with a relatively small percentage of

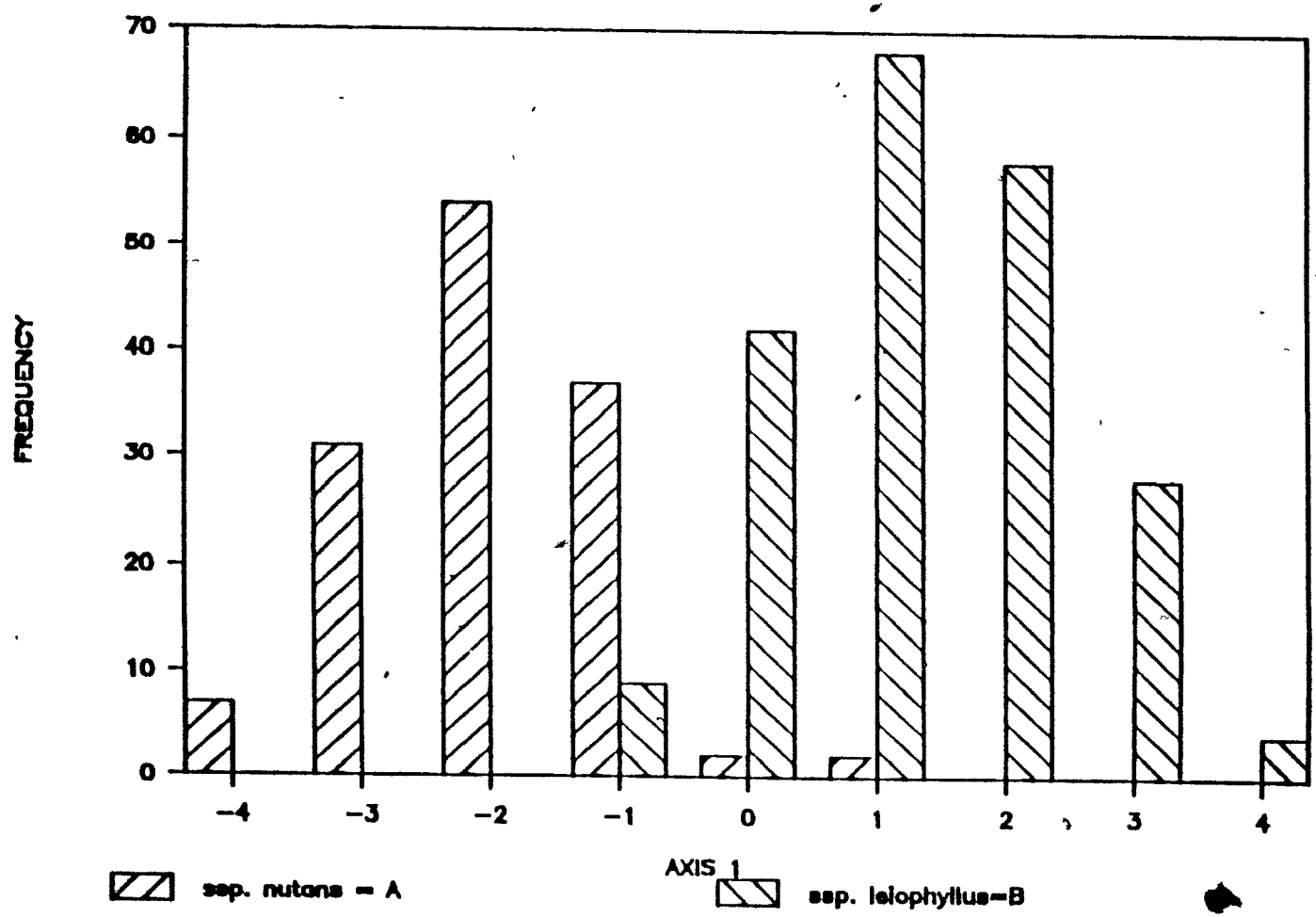

FIGURE 3 - Histogram of the frequency distribution of the first canonical variate values in a comparison of individuals of ssp. nutans with ssp. leiophyllus of Carduus nutans.

overlap. A discriminant analysis performed on the two groups indicated that the correct classification rates of individuals in group A was of 93.7 % (118/126) and that 96.0% (190/198) of group B individuals were correctly assigned to group B. Group A includes individuals from seven populations which were all collected in New Brunswick and Ontario. Group B includes individuals from 11 populations: one collected in Québec (#297), one population in Ontario (#250), while the remaining nine were collected in the provinces or states west of Ontario. Based on the examination of herbarium specimens and the descriptions provided in previous treatments, the individuals in group A and B are recognized to be members of ssp. nutans and ssp. leiophyllus, respectively.

The results obtained for the qualitative and quantitative measurements recorded on the herbarium specimens are presented in Tables 5 and 7 respectively. Table 7 compares the means of the head diameter (HDD) and phyllary width above the constriction (PWMT) between the herbarium and population samples. For both the herbarium specimens and population samples, the means obtained for the two characters are lower in ssp. nutans than in ssp. leiophyllus. Since the range of the head diameter (HDD) and the range and mean for phyllary width (PWMT) fall within the values obtained for ssp. leiophyllus, specimens labeled as

Table 7 - Comparison of the means, minimum and maximum values obtained for the quantitative morphological measurements of herbarium specimens and population samples.

	HEAD DIAMETER (HDD)		PHYLLARY WIDTH (PWMT)	
	MEAN	MIN - MAX	MEAN	MIN - MAX
Herbarium data				
ssp. nutans[1]	2.36	1.50 - 3.50	0.29	0.10 - 0.50
S.E.	0.50		0.10	
ssp. leiophyllus	2.90	1.80 - 4.50	0.49	0.25 - 0.80
S.E.	0.70		0.58	
ssp. macrolepis[2,3]	3.07	2.30 - 3.80	0.44	0.35 - 0.50
S.E.	0.67		0.19	
ssp. leiophyllus and macrolepis	2.94	1.80 - 4.50	0.48	0.25 - 0.80
S.E.	0.69		0.03	
Population samples data[3]				
ssp. nutans	(1.89)[4]	(1.15 - 3.05)	0.32	0.15 - 0.50
S.E.	0.03		0.006	
ssp. leiophyllus	(2.32)	(1.45 - 3.50)	0.45	0.20 - 0.80
S.E.	0.03		0.008	

[1] n=33-39 for ssp. nutans and ssp. leiophyllus

[2] n=4

[3] n=126 for ssp. nutans and n=198 for ssp. leiophyllus

[4] () head diameter was measured differently on the population samples (see materials and methods)

ssp. macrolepis were included with ssp. leiophyllus. When this was done the mean values for ssp. leiophyllus did not change significantly.

The results obtained for the qualitative characters recorded on herbarium specimens fall well within the range of percentage obtained for the population samples (Table 5).

Electrophoretic Analysis

Nine enzyme systems were assayed for all populations. Malate dehydrogenase (MDH) was scored for all populations but could not be readily interpreted because of overlap of isozymes. This enzyme is reported in Table 8 as phenotypic patterns; it was not included in the calculation of genetic identities or genetic variability. The remaining eight enzymes are coded by 16 loci. Two of these loci, Idh-1 and 6-Pgd-1, were not recorded due to the poor resolution obtained.

Very little variation in allozyme frequency was evident in the enzyme systems studied (Table 8). Seven gene loci, Tp1-2, Pg1-1, G-3pd-1 and G-3pd-2, Me and Pgm-1 and Pgm-2 were monomorphic for identical alleles for all plants. One allele, Pg1-2a, was detected in five populations of ssp. leiophyllus and Idh-2c was unique to population #280 of ssp. leiophyllus. No correlation between these results and the morphological and flavonoid results could be made. Genetic identities for all pair-wise comparisons of populations and

TABLE 8 - Allelic frequencies in 19 populations of Carduus nutans.

Loci		231	232	243	245	246	248	249	250	252	256	260	261	267	269	273	280	285	297	BC
Tpi	1a	0.82	1.0	0.98	1.0	1.0	0.99	0.98	1.0	1.0	1.0	1.0	0.79	0.65	0.8	0.89	0.8	0.98	1.0	1.0
	1b	0.18		0.02			0.01	0 02					0.21	0.35	0.2	0.11	0.2	0.02		
	2a																			
	2b	1.0	1.0	1 0	1.0	1.0	1.0	1.0	1.0	1.0	1 0	1.0	1.0	1 0	1.0	1.0	1.0	1.0	1.0	1.0
Pgi	1a	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1 0	1.0	1 0	1 0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	1b																			
	2a												0 13		0.02	0 09	0.13	0.02		
	2b	1.0	1.0	0.98	1.0	0.94	0.84	0 86	0.87	0.74	0.69	0.85	0.34	0 52	0.25	0.3	0.45	0.98	0.69	0.54
	2c			0 02		0.06	0 16	0.14	0.13	0.26	0.31	0.15	0.53	0.48	0.73	0.61	0.42		0.31	0.46
Got	1a	0.94	0.93	0.94	0.95	0.73	0.98	0.7	0.73	0.15	0 45	0.45	0.09	0.28	0.48	0 41	0.62	0.26	0.1	1.0
	1b	0.06		0.06	0.05	0.21	0.02	0 21	0 17	0 85	0.32	0.3	0 88	0 72	0 52	0 51	0.26	0.29	0.13	
	1c		0.07			0.06		0.09	0 1		0 23	0.25	0.03			0.08	0.12	0.45	0.77	
	2a	1.0	1 0	1.0	1.0	0 92	0 96	0 86	0.62		0.89	0.77	0.74	0 87	0.95	0.85	0.64	0.53	0.65	1.0
	2b					0.08	0.04	0.14	0.38	1 0	0.11	0.23	0.26	0 13	0 05	0.15	0.36	0.47	0.35	
	3a	0.25	0.86	0.35	0.67	0.27	0 22	0.39	0 19		0 39	0.41	0.29	0.52	0 89	0.54	0.39	0.32	0.31	0.71
	3b	0.75	0.14	0.65	0.33	0.73	0.78	0.61	0 81	1.0	0.61	0 59	0.71	0 48	0.11	0.46	0.61	0.68	0.69	0.29
Idh	2a	0.29	0.4	0.5	0.41	0.45	0 46	0.44	0 25	0 45	0.48	0.38	0.24	0.42	0.25	0.18	0.24	0.39	0.19	
	2b	0.71	0.6	0.5	0.59	0.55	0.54	0.56	0 75	0 55	0.52	0.62	0.76	0 58	0 75	0 82	0 64	0.61	0.81	1.0
	2c																0.12			
6-pgd	2a	1.0	0.97	1.0	1.0	0 85	0.7	0.76	0.44	0 74	0.42	0.17	0.93	1.0	0.62	0.66	0.51	0.4	0.25	0.65
	2b		0.03			0.15	0.3	0 24	0 56	0.26	0 58	0.83	0 07		0.38	0.34	0.49	0.6	0.75	0.35
G-3pd	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1 0	1 0	1 0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
G-3pd	2	1.0	1.0	1.0	1.0	1.0	1 0	1.0	1 0	1 0	1.0	1 0	1 0	1 0	1.0	1.0	1.0	1.0	1.0	1.0

Mp - All individuals monomorphic for the locus
PGM - All individuals monomorphic for the 2 loci

*MDH	1.0	32	44	49	34	54	17	14	03										
	2	5	16	17	15	31	77	69	32	92	73	97	1 0	7	6	19	97	3	75	1.0
	3		9	8	15	6	3	14	3					13	4	47			4	
	4	63	25	23	36	8	3		5	4				17		34				
	5								11	4	4	3					3			
	6		6	3		2			3		19							57	18	
	7								16		4							13	3	

*Results presented as phenotypic patterns, in percentage.

mean genetic identities within and between subspecies of C. nutans are presented in Tables 9 and 10, respectively. These results fall within the range of values expected for conspecific populations and/or subspecific taxa for outcrossing plants (Crawford, 1983; Gottlieb, 1977, 1981b). In general, both subspecies have similar frequencies for shared alleles suggesting that little or no genetic differentiation between the taxa has occurred.

The measures of genetic variability (Table 11) show similar results among the populations and fall within the range of values expected for outcrossing plants as presented by Gottlieb (1977). Five populations (#231, #232, #243, #245, and BC) have values of proportion of polymorphic loci slightly lower than expected. This may reflect the fact that these populations are from more isolated locations where possibly a more limited number of seeds were introduced. In ssp. nutans the mean value for the proportion of polymorphic loci is 0.41 and for ssp. leiophyllus, 0.46. The mean number of alleles per polymorphic locus is 2.08 for ssp. nutans whereas ssp. leiophyllus has a value of 2.17. The mean observed heterozygosity values for ssp. nutans and ssp. leiophyllus are 0.09 and 0.16 respectively. The expected heterozygosity values are 0.10 for ssp. nutans and 0.18 for ssp. leiophyllus.

TABLE 9 - Genetic Identities among 19 populations of Carduus nutans

	231	232	243	245	246	248	249	250	252	256	260	261	267	269	273	280	285	297	BC
231	1.00																		
232	0.97	1.00																	
243	0.99	0.98	1.00																
245	0.98	1.00	0.99	1.00															
246	0.99	0.97	0.99	0.98	1.00														
248	0.99	0.96	0.99	0.97	0.99	1.00													
249	0.98	0.97	0.99	0.98	1.00	0.99	1.00												
250	0.96	0.92	0.95	0.94	0.97	0.98	0.98	1.00											
252	0.85	0.80	0.85	0.83	0.89	0.87	0.90	0.92	1.00										
256	0.94	0.94	0.95	0.94	0.97	0.97	0.98	0.98	0.89	1.00									
260	0.92	0.91	0.92	0.92	0.95	0.95	0.96	0.98	0.89	0.99	1.00								
261	0.90	0.86	0.89	0.88	0.92	0.89	0.92	0.90	0.92	0.93	0.89	1.00							
267	0.94	0.92	0.94	0.93	0.95	0.93	0.95	0.91	0.89	0.94	0.91	0.97	1.00						
269	0.89	0.92	0.90	0.92	0.91	0.91	0.93	0.90	0.82	0.95	0.92	0.93	0.96	1.00					
273	0.92	0.92	0.92	0.92	0.94	0.94	0.96	0.94	0.88	0.97	0.94	0.97	0.97	0.98	1.00				
280	0.94	0.92	0.94	0.93	0.96	0.96	0.97	0.98	0.91	0.98	0.97	0.94	0.95	0.96	0.98	1.00			
285	0.92	0.91	0.92	0.91	0.95	0.94	0.96	0.98	0.93	0.98	0.98	0.90	0.91	0.89	0.93	0.96	1.00		
297	0.88	0.87	0.88	0.87	0.91	0.91	0.93	0.96	0.89	0.97	0.97	0.89	0.88	0.89	0.93	0.95	0.98	1.00	
BC	0.95	0.96	0.95	0.96	0.95	0.96	0.96	0.94	0.80	0.95	0.93	0.89	0.92	0.96	0.96	0.96	0.90	0.90	1.00

Table 10 - Mean genetic identities (I) within and between subspecies of Carduus nutans.

	I	RANGE
ssp. nutans	0.98	0.96 - 1.00
ssp. leiophyllus	0.93	0.80 - 0.99
between subspecies	0.93	0.80 - 0.98

TABLE 11 - Proportion of polymorphic loci, mean number of alleles per polymorphic locus, and heterozygosity, for 19 populations of Carduus nutans.

Pop.	Polymorphic loci 99%	Mean # of alleles	Heterozygosity Obs.	Exp.
nutans				
231	0.36	2.00	0.06	0.08
232	0.29	2.25	0.06	0.07
243	0.36	2.00	0.08	0.09
245	0.29	2.00	0.07	0.07
246	0.50	2.17	0.11	0.11
248	0.50	2.00	0.12	0.12
249	0.57	2.13	0.16	0.17
leiophyllus				
250	0.50	2.10	0.15	0.16
252	0.29	2.00	0.11	0.12
256	0.43	2.17	0.16	0.20
260	0.43	2.17	0.16	0.18
261	0.50	2.29	0.15	0.17
267	0.43	2.00	0.14	0.18
269	0.50	2.14	0.13	0.17
273	0.50	2.29	0.18	0.20
280	0.50	2.43	0.21	0.24
285	0.50	2.14	0.20	0.19
297	0.43	2.17	0.17	0.17
BC	0.21	2.00	0.09	0.10

Flavonoid Analysis

Six flavonoid glycosides were isolated from the C. nutans complex. The spectral and R_f data are presented in Table 12. The distribution of the flavonoids in the 19 populations is given in Table 13. The different flavonoid profiles observed for each individual in the within population variation survey are presented in Fig. 4, and were designated as either P1, P2, or P3. The relative number of profiles recorded per population are presented in Table 14. Only two flavones, apigenin and luteolin, were isolated. The identification of one glycoside (luteolin 7-O-monoglucoside) is tentative due to the poor resolution obtained on the chromatogram. However, the R_f values obtained for this compound match the ones presented in previous reports (Harborne, 1967). The variation observed between the populations resides in the type of aglycone, and the type and number of sugars. Three of the six compounds isolated are present in all populations. One compound (luteolin 7-O-rutinoside) characterizes ssp. nutans while two compounds (apigenin 7-O-glucoside and luteolin 7-O-diglucoside) are found in all populations of ssp. leiophyllus and in three populations of ssp. nutans. The survey of the within population variation revealed that the two latter compounds are only found in some individuals in these three populations. With respect to the third profile the absence of 7-O-glucoside may reflect a lower quantity of this compound.

TABLE 12 - Spectral and Rf data for the flavonoids of Carduus nutans.

COMPOUND	SPECTRAL SHIFTS (Compared to MeOH)							Rf[a] X 100				COLOURS[b] (at 350 nm)		
	max in 100% MeOH		NaOMe	$AlCl_3$	$AlCl_3$/HCl	NaOAc	H_3BO_3							
	Band II	Band I	Band I	Band I	Band I	Band II	Band I	BAW	H_2O	HoAc	PhOH	UV	UV/NH3	UV/NA[c]
Luteolin	255, 268	351	+51	+73	+38	+13	+3	66	1	5	66	P	YG	Y
Luteolin 7-O monoglucoside[d]	256	346	+44	+81	+44	+0	+17	26	4	23	55	P	Y	Y
Luteolin 7-O diglucoside	254, 268s[a]	344	+38	+82	+46	+3	+21	38	6	40	79	P	Y	Y
Luteolin 7-O galactoside	256, 280s	347	+34	+80	+39	+4	+38	42	2	15	64	P	Y	Y
Luteolin 7-O rutinoside	253	351	+53	+72	+39	+6	+21	41	4	28	58	P	Y	Y
Apigenin 7-O glucoside	270	342	+53	+43	+43	+0	+3	42	7	49	89	P	YG	G

[a] s, shoulder.

[b] Solvent composition : BAW, 1-butanol:acetic acid:water (4:1:5, upper phase); HoAc, 15% acetic acid; PhOH, 85% phenol.

[b] Colour key: G, green; P, purple; Y, yellow; YG, yellow-green.

[c] NA, Naturstoffreagens A in MeOH.

[d] sugar identification based on Rf values from glycoside only.
- see text for details.

TABLE 13 - Distribution of flavonoids in populations of Carduus nutans

	ssp. nutans							ssp. leiophyllus											
	231	232	243	245	246	248	249	250	252	256	260	261	267	269	273	280	285	297	BC
COMPOUNDS*																			
A 7-O-glycoside					(+)	(+)	(+)	+	+	+	+	+	+	+	+	+	+	+	+
Luteolin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L 7-O-monoglucoside	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L 7-O-diglucoside					(+)	(+)	(+)	+	+	+	+	+	+	+	+	+	+	+	+
L 7-O-galactoside	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L 7-O-rutinoside	+	+	+	+	(+)	(+)	(+)												

* Compounds: A, apigenin; L, luteolin
() Compounds found in some individuals only

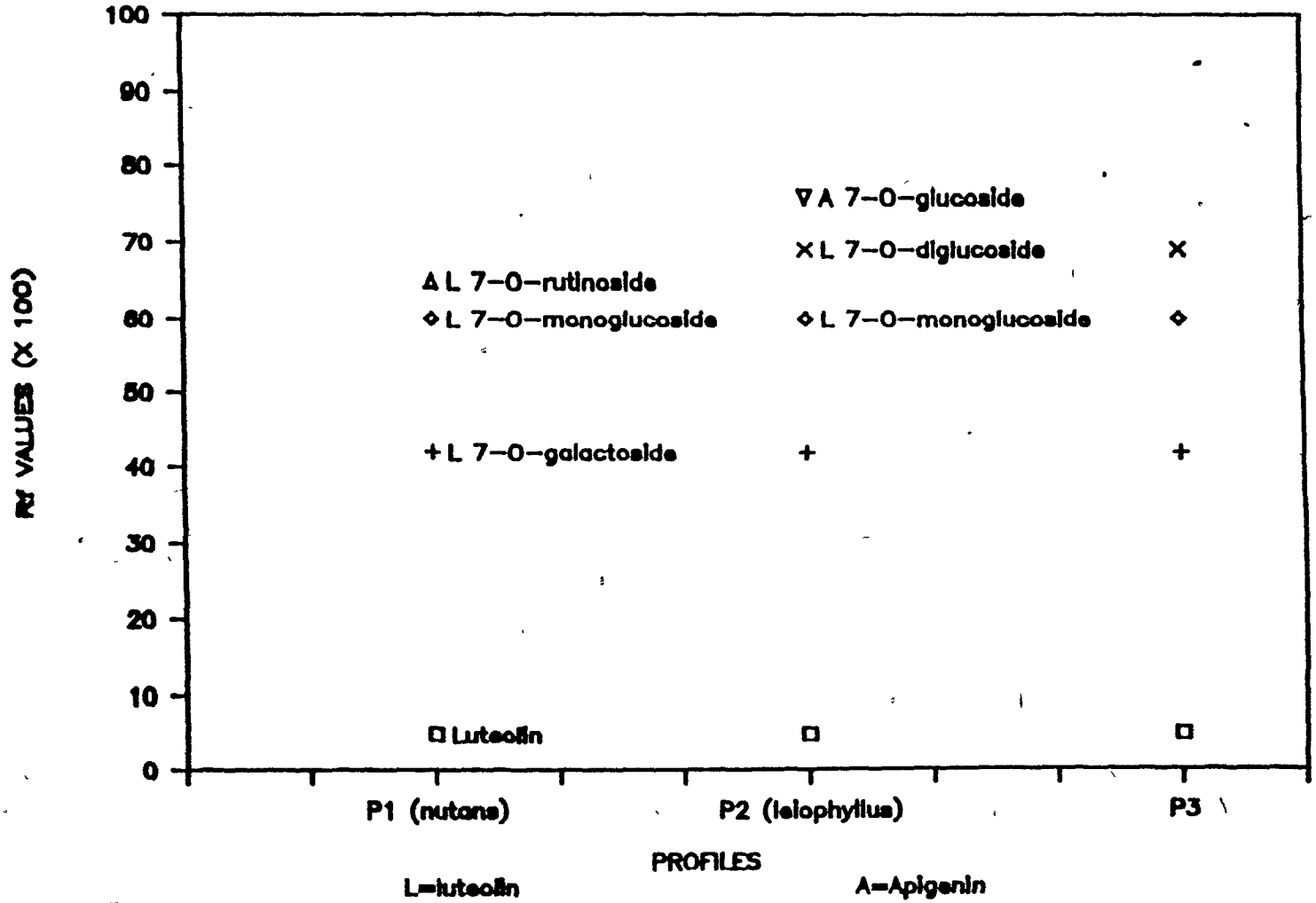

FIGURE 4 - Flavonoid profiles on TLC observed in a survey of within population done on the individuals of 19 populations of Carduus nutans. (P1 - ssp. nutans, P2 - ssp. leiophyllus, P3 - profile observed only in some individuals of populations #246, #248, #249.)

TABLE 14 - Number of individuals per flavonoid profile for 19 populations of Carduus nutans.

POPULATIONS	PROFILES 1	2	3
231	14		
232	16		
243	26		
245	20		
246	16	1	4
248	5	7	7
249	10	2	3
250		19	
252		13	
256		17	
260		17	
261		18	
267		16	
269		18	
273		17	
280		17	
285		18	
297		18	
BC		18*	

* Some individuals had a flavonoid compound only found in that population.

Based on spot intensity, apigenin 7-O-glucoside was the compound present in the lowest quantity. Since profiles were generated using a minimum of leaf material the compound may still be present in profile 3 but in undetectable amounts.

DISCUSSION

The results obtained from the analyses performed support the recognition of two closely related taxa best treated at the subspecific level; these are C. nutans ssp. nutans and ssp. leiophyllus.

Both the morphological and the flavonoid analyses clearly indicate the existence of only two taxa. The classificatory discriminant analysis showed that the taxa separated well, with an overlap of less than 6% when all 15 characters were considered simultaneously. However, for the two subspecies each of the 15 characters shows considerable within subspecies variation and some degree of overlap between subspecies. This probably explains why, when the 18 populations were used in the canonical analysis, they failed to form morphologically distinct groups.

Since the variation between the taxa is continuous for many characters, they are best distinguished by using a combination of characters, including; presence/absence of pubescence on the plants, head diameter, and phyllary width, both above and below the constriction. The best taxonomic character for separating the two subspecies is the presence/absence of pubescence on the plants. This character has been previously used by Moore and Frankton (1974) to separate ssp. nutans from the two other subspecies they recognized, ssp. leiophyllus and ssp. macrolepis. According

to Moore and Frankton (1974) the latter two subspecies could be separated from one another based on the shape of the apical portion of the phyllary. In the present study this character was scored on herbarium specimens and population samples and was found to be nearly uniform throughout the populations. Differences were noticed on some specimens but they were not deemed sufficient to separate any taxa. This character was often found to be variable on the same individual. In the absence of any reliable method for separating ssp. macrolepis from ssp. leiophyllus, ssp. macrolepis is not recognized here.

Of the six compounds identified in the flavonoid analysis, three were nearly diagnostic; apigenin 7-O-glucoside and luteolin 7-O-diglucoside were found in all populations of ssp. leiophyllus, and luteolin 7-O-rutinoside characterized ssp. nutans. The two flavonoid compounds that were present in all populations of ssp. leiophyllus, were also isolated from some individuals in three Ontario populations of ssp. nutans (two from Grey Co., one from Halton Co.). The sporadic occurrence of this compound in ssp. nutans suggests that hybridization between the two subspecies may be occurring in these regions. Both infraspecific (McCarty et al., 1980; Moore and Frankton, 1974) and interspecific (Moore and Mulligan, 1956, 1964; Mulligan and Moore, 1961; Warwick and Bain, 1987) hybridization involving C. nutans has been

previously reported. Furthermore, these three populations are located in a region where the distributions of the two subspecies overlap (see Fig. 5).

The results obtained from the isozyme analysis do not allow the distinction of any taxa in the C. nutans complex. Very little variation in allelic frequency was noticed among the populations. The mean genetic identities between the populations of each subspecies are essentially similar to the mean genetic identity obtained between the two subspecies (Table 11). Similar results have been reported in other studies where infraspecific taxa have been examined electrophoretically (Crawford and Smith, 1984, McLeod et al., 1983; Soltis, 1982; van Dijk and van Delden, 1981). In all cases the mean genetic identities of the taxa were above 0.90 as were those obtained in this study.

Our treatment of the C. nutans complex in Canada differs from that of Moore and Frankton (1974). Both treatments provide similar descriptions of ssp. nutans but the descriptions of ssp. leiophyllus are different. These differences mainly reside in the size of the head and of the phyllary. Measurements of head and phyllary size provided by Moore and Frankton (1974) are greater in individuals of ssp. leiophyllus and the overlap between ssp. nutans and ssp. leiophyllus is smaller. In the present study very few head and phyllary measurements were as large as the ones presented

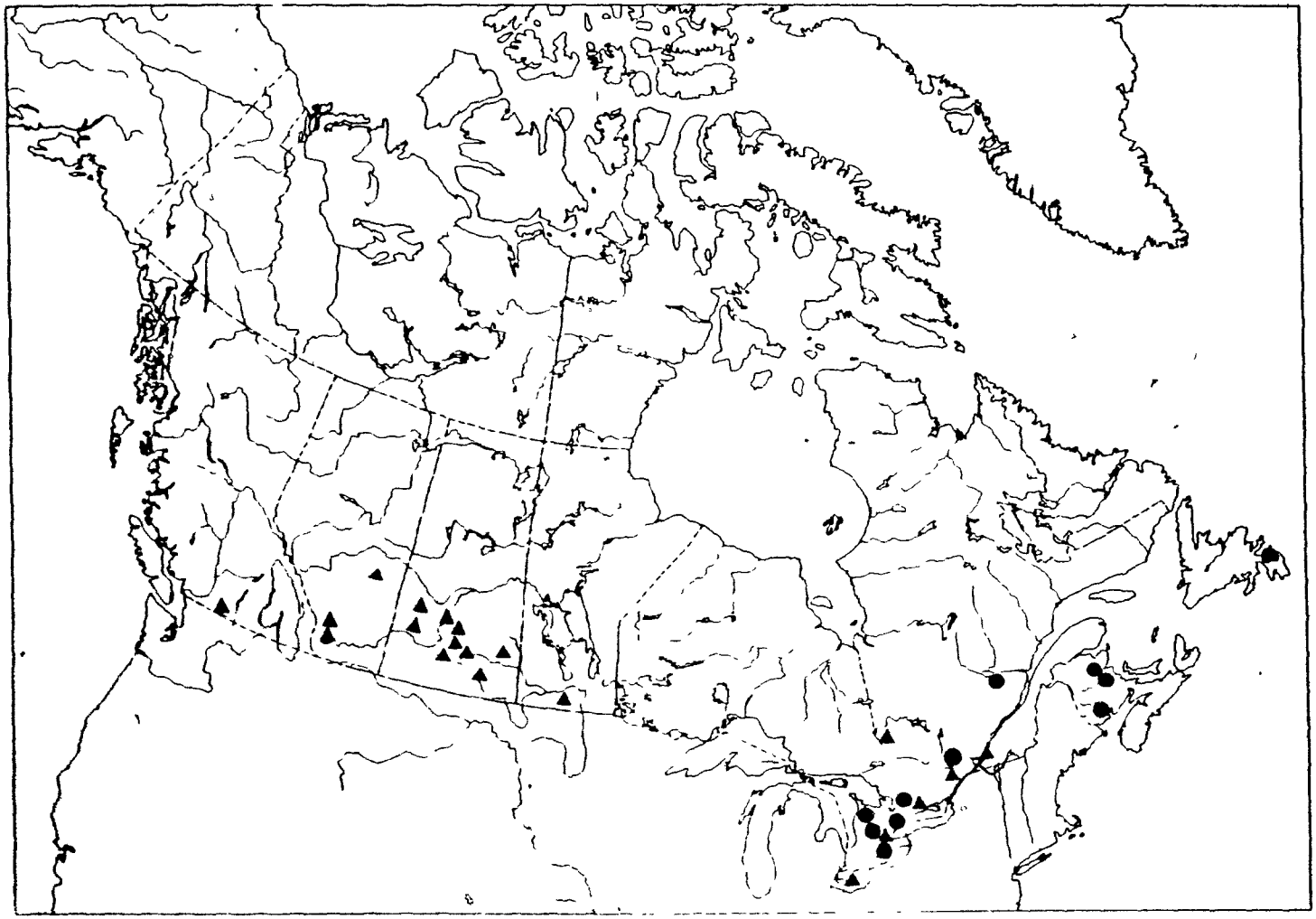

FIGURE 5 - Distribution of Carduus nutans in Canada.
(● ssp. nutans; ▲ ssp. leiophyllus)

by Moore and Frankton (1974). Limited sample size and variation among heads on a plant may well explain the discrepencies found between the measurements provided in the present study and those by Moore and Frankton (1974).

The present treatment also differs from those of Boldt (1978) and McCarty (1978) who recognized ssp. leiophyllus at the specific level (as C. thoermeri) rather than at the subspecific level. Their treatments were based largely on specimens from the United States. Since all the United States specimens examined, including population samples from North Dakota and Montana and herbarium specimens, fit well into the present circumscription of ssp. leiophyllus we conclude that any disagreement among the treatments is based on rank. In a recent paper presenting limited data gathered from nursery studies, McCarty (1985) also suggested that C. thoermeri may be better treated at the subspecific rank.

In McGregor's (1986) treatment of Carduus, only two subspecies are recognized, ssp. macrocephalus and ssp. leiophyllus, in the area covered by the Flora of the Great Plains. The northern limit of this area extends into the provinces of Saskatchewan and Manitoba and with respect to ssp. leiophyllus our two treatments are largely in agreement. McGregor's description of ssp. leiophyllus indicates that on the whole individuals of this taxon are slightly larger than those measured in this study, but no other major differences are apparent. The size differences may reflect a normal range

of phenotypic plasticity. Herbarium specimens of ssp. macrocephalus annotated by McGregor were examined. This taxon is clearly distinct from ssp. leiophyllus and so far no specimens of ssp. macrocephalus have been collected in Canada.

McGregor's finding that C. nutans ssp. nutans does not occur on the Great Plains is in agreement with the findings of this study. In Canada, this taxon tends to be more common in the eastern provinces (Fig.5) and presumably ssp. nutans shows a similar distribution pattern in the United States. The distribution of ssp. nutans in Canada covers Ontario and some of the provinces east of Ontario. Although predominantly western in distribution, populations of ssp. leiophyllus are also present in Ontario and Québec. Dunn (1976) illustrated the distribution of C. nutans in the United States but the distributions of subspecies were not shown.

Clearly, most of the discrepancies between the North American treatments of the C. nutans complex relate to the differing views of the variation found in ssp. leiophyllus. The European treatments (i.e. Franco, 1976 and Kazmi, 1964) are in agreement in treating ssp. leiophyllus as C. thoermeri. Although their descriptions are similar to ours it is unclear whether the two taxa are the same.

When predicting the genetic structure of populations several factors must be considered, but two may be particularly important when dealing with introduced weeds. They are the breeding system of the species and the number of

introductions which have occurred. With respect to the former factor, outcrossing species are predicted to have most of their genetic variation occurring within the populations rather than between the populations when compared with inbreeders or selfers (Hamrick et al. 1979 and Loveless and Hamrick, 1984). C. nutans is predominantly outbreeding and the electrophoretic data presented suggests that it is behaving in a predictable fashion, in as much as our results show no evidence of reduced variability.

The actual introduction of C. nutans to North America is not well documented but evidence from herbarium specimens and other sources suggests that a number of separate introductions have occurred. The first collections of the species were from several ballasts along the east coast of North America (Stuckey and Forsyth, 1971). These specimens represent individuals of ssp. nutans. Since ssp. nutans was first collected in the United States it is unclear whether this taxon was introduced in Canada from seeds originating from the United States or Europe. Harris (unpubl.) believes that ssp. leiophyllus was separately introduced to Alberta, Saskatchewan, and Manitoba via seeds originating from the United States, while Mulligan and Frankton (1954) speculated that this taxon was introduced in Saskatchewan from seeds originating from Argentina. The first collections of C. nutans in the mid or western regions of the United States are

sporadic suggesting that there may also have been a number of separate introductions in these regions. If separate introductions have occurred, little genetic differentiation between European and North American populations should be expected.

Although this study has provided additional information to the previous treatments of this complex in Canada, we are unable, from the results of this study, to determine with certainty whether C. thoermeri (sensu Kazmi) and C. nutans ssp. leiophyllus are the same. A more detailed morphological and genetic study involving a comparison of the populations from both continents would help clarify the remaining problems.

TAXONOMIC DESCRIPTION AND KEY

Carduus nutans L., nodding thistle, musk thistle.

Herbaceous annual or biennial, 2 to 20 dm tall, glabrous or pubescent on the leaves and phyllaries. Stem erect, single or 5 or 6, usually much branched, with spiny triangular or palmate wings, glabrous or moderately pubescent. Basal rosette well developed, leaves elliptic to lanceolate, 15 to 30 cm long, glabrous to densely pubescent, pinnatifid with palmate or triangular lobe, lobe ending in a spine. Cauline leaves simple, alternate, sessile, decurrent, lanceolate or oblong-lanceolate, glabrous to densely pubescent, pinnately lobed with numerous small marginal spines, lobe tapering to a sharp spine. Head terminal, solitary, peduncle naked or with a few small bracts for 1.5 to 5(7) cm below the head, or on flower head maturing later in the season often leafy below. Head 1.5 to 4.5(7) cm in diameter, usually nodding; phyllaries numerous imbricate, 9 to 27 mm long, reflexed or spreading, tapering to a spine (1)2 to 4 mm long, middle row phyllary with a constriction at or slightly below the mid point, portion above oblong to lanceolate, wider than or equal to the lower portion, inner phyllaries narrow, unarmed. Flower perfect, tubular, pink to purple; pappus 15 to 20(25) mm long, corolla

tube 10 to 14 mm long, anther 6.0 to 8.5 mm long. Achene 3.2 to 5.0 mm long, 1.4 mm wide. n=8. Growing in pastures, along roadsides, and waste fields.

Key to the subspecies

Phyllaries with arachnoid hairs, lower portion equal to or slightly narrower than upper portion; leaves moderately to densely pubescent; head diameter 1.5-3.5 cm ..ssp. nutans

Phyllaries glabrous, lower portion definitely narrower than upper portion; leaves glabrous to slightly pubescent; head diameter 1.8-4.5(7)cm .....ssp. leiophyllus

Carduus nutans L. ssp. nutans* Sp. Pl. 821. 1753.

Stem and leaves moderately to densely pubescent. Head diameter 1.5-3.5 cm. Phyllary with arachnoid hairs, upper portion 10-19 mm long, 2.5-4.1 mm wide, lower portion 6.0-8.0 mm long. 2.0-3.0 mm wide, constriction not always obvious. Achene 3.2 to 3.8 mm long, 1.4 mm wide. Widespread in Ont., occurring in N.B., Nfld and Que., reported to occur in N.S. but no specimens have been cited or seen.

REPRESENTATIVE SPECIMENS

New Brunswick. : Bass River, NE of Harcourt H.J. Scoggan & D.S. Erskine 12910 (CAN 238655, QFA 124546) ; Chatham H.J. Scoggan 12125 (CAN 238659) ; Chatham H.J. Scoggan 13352 (ACAD 50241, SHER 20900) ; Bass River J. Fowler 337 (ACAD s.n.) ; Chatham (3 mi S.) W.G. Dore & E. Gorham 45551 (QFA 276737, ACAD 16355) ; Northumberland Co.: Newcastle, H.J. Scoggan 13350 (ACAD 50515, CAN 243523) ; Newcastle-Douglastowm, H.R. Hinds 6214 (UNB 42317) **Ontario.** : Kitchener, C.A. Campbell & G.R. Donaldson 74-128 (CAN 377238) ; Southampton, G.A. Mulligan & D.R. Lindsay 874 (DAO 384081) ; Bruce Peninsula, J. Simon 389 (DAO 384083) ; Brant Co.: Paris H.J. Scoggan 14449 (CAN 306882) ; Carleton Co.: Betweem Conc. 6 & 7 I.J. Bassett et al. 2331 (DAO 384108) ; Frontenace Co.: Fermoy G.A. Mulligan & W.G. Dore 907 (DAO 384084) ; Grey Co.: Maxwell, Artemisia Twp, D.R. Lindsay & G.A. Mulligan 275 (DAO 384096) ; Durham (3 mi W), G.A. Mulligan & D.R. Lindsay 863a (DAO 393993) ; Durham (3 mi W), G.A. Mulligan & D.R. Lindsay 863c (DAO 393992) ; Priceville, G.A. Mulligan & R.J. Moore 1000 (DAO 39027) ; Priceville, G.A. Mulligan & R.J. Moore 1015 (DAO 384104) ; Halton Co.: Milton, G.A. Mulligan & D.R. Lindsay 817 (DAO 384087) ; Acton, R.F. Cain 1518 (SHER 69926, QFA 276738) ; Campbellville, Z.D. Bezdek 4287 (TRTE 8014) ; Leeds Co.: Portland (5 mi NE), G.A. Mulligan & W.G. Dore 908

(DAO 384102) ; Oxford Co.: Tavistock s.n. (DAO 384090) ; Peel Co.: Meadowvale, A.F. Coventry 64/42 (TRTE 645) ; Inglewood, A.F. Coventry 64/62 (TRTE 643) ; Cheltenham, Conc. 5, lot 32, H. Saifi et al. 9461 (DAO 208508) ; Mississauga, J.M. Webber 3023 (3) (DAO 281735, TRTE 17841, CAN 452015) ; Perth Co.: Brocksden T. McIntosh s.n. (SHER 153803) ; Victoria Co.: Beaverton I.J. Bassett 3357 (DAO 384088) ; Waterloo Co.: Galt, C. Frankton, et al. 650 (ALTA 68210, DAO 384091) ; Preston, H. Groh 3281 (DAO 384092) ; Grand River, Preston, H. Groh 3441 (DAO 384093) ; Wellington Co.: Guelph, E.G. Anderson 1811 (MIN 490802, DAO 39030) ; Wentworth Co.: Westover, Beverly Tp. J.E. Cruise et al. 9461 (QUE 41468) ; York Co.: Humber River (E side) J Laudenbach 45373 (TRTE 9468) **Quebec.** Lac St-Jean Co.: St-Jerome, A. Belzile & C. Gervais 2740 (DAO 384038, QK 92223, SHER 4133, QFA s.n.) ; Ste-Croix, Caron Tsp, I.J. Bassett & A. Hamel 2135 (DAO 384042) ; St-Jerome, I.J.Bassett 2082 (QUE 6101) ; St-Gedeon, J. Cayouette 73-474 (SHER 114885, QFA 143615, QUE 63776) ; Ste-Croix, L. Bergeron 103-25-1 (QFA 120046) ; St-Jerome, L. Cinq-Mars et al. 547 (QFA 144692) ; St-Jerome, L. Cinq-Mars et al. 63-1162 (QUE 261,08, QFA 276739, QFA 76780, ACAD 6319,QFA 72873) ; Desbiens, R. Caouette & J.-C. Michel 6682 (DAO 384046) ; St-Jerome R. Cayouette & C. Leduc 8959 (QUE 33991) ; Desbiens, R. Cayouette & J-C. Michel 6682 (SHER 21067, QUE 20531) ; Quebec

Co.: Ste-Foy, Cte Quebec C. Rousseau 64-481 (QUE 77012, QFA 76779, SHER 7575, QUE 33433) **Newfoundland,** : Near Heart's Content, Avalon Peninsula I.J. Bassett 617 (DAO 384052).

Carduus nutans ssp. leiophyllus* (Petrovic) Stoj. & Stef., Fl. Bulg. 3rd ed. 1183. 1948.

C. nutans var. leiophyllus (Petrovic) Arenes sensu Mulligan & Frankton, Can. Field Nat. 68: 35 (1954)

C. nutans var. vestitus (Hal.) B. Boivin. Nat. Can. 94: 654 (1967)

C. thoermeri Wienm sensu McCarty, Biological control of thistles in the genus Carduus in the United States, p.9 .1978.

Stem and leaves glabrous to slightly pubescent. Head diameter 1.8-4.5(7) cm wide. Phyllary glabrous to slightly pubescent, upper portion 10-16 mm long, 2.5-8.0 mm wide, lower portion 6.0-8.0 mm long, 2.5-3.6 mm wide, constriction obvious. Achene 4.0-5.0 mm long, 1.4 mm wide. Widespread in Sask., occurring in Alta, B.C., Man., and Que..

* Kazmi (1964) provides lists of synonyms for ssp. nutans and ssp. leiophyllus that have been used in the Old World.

REPRESENTATITE SPECIMENS

Alberta. : Beddington (Calgary) B.M. Hallworth & D. Allen s.n. (UAC 26723, ALTA 86109) ; Beddington (Calgary) C. Osborne

s.n. (UAC 26727, UAC 26726, UAC 26724) ; Beddington (Calgary) C. Osborne s.n. (CAN 400658, UAC 26725, DAO 145415) ; Calgary L.V. Hills s.n. (QK 126933, UNB 41404, UAC 40228) ; Calgary M. Mychajluk 1009 (UNB 37289, UAC 40227, QK 126932) ; Claresholm R. Berringer s.n. (DAO 282126) **Manitoba.** : Reinland F.G. Ens E39 (DAO 659569) ; Pembina L. Young s.n. (DAO 653208) ; Haywood W.G. Dore & A.J. Breitung 12727 (DAO 281690) Thornhill H.H.Marshall M1661 (DAO 160766) **Ontario.** Essex Co.: Pelee Island M.J. Oldham 3901 (TRTE 33615) ; Hastings Co.: Marmora (10 mi S) K.J. Crawford 547 (QK 106488) ; Roslin, Lot 3 and 4 Conc. 9 G.A. Mulligan & V.R. Paxton 952 (DAO 384068) ; Lennox-Addington Co.: Odessa (3 mi N) K.J. Crawford 584 (QK 106490) ; Odessa (3 mi N) K.J. Crawford 594 (QK 106348) ; Northumberland Co.: Potts Island, Brighton Twp, J.L. Riley & D.Hoy 9461 (DAO 199400) Peterborough Co.: Norwood C. Frankton et al. 630 (DAO 384067) ; Peterborough S.L. Gray & M.N. Campbell 1595C (CAN 380776) **Quebec.** : Cte Temiscamingque : Ville Marie, G. Sirois s.n. (DAO 384069) ; Montréal D. Woodland 2134 (MIN 702493) ; Montreal-Est R. Neron 84-245 (QUE 93138) **Saskatchewan.** : Swift Current A.C Budd 2516 (UAC 26729) : Craik to Girvin, Lake Centre District B. Boivin & D. Dunbar 10308 (DAO 126497, DAO 126495, WIN 32528) ; Craven B. Boivin & W.G. Dore 7567 (ALTA 73449, SASK 64310, DAO 126496) ; Eyebrow, District de Moose Jaw B. Boivin et al. 11563 (DAO 126480, REG s.n.) ; Elbow, along camp road B. de Vries 1083.69 (REG 8626) ; Souris creek, east of Lewvan B. de Vries 28-70

(REG s.n.) ; Melville, Hwy 10 B. de Vries 3581.75 (REG s.n.) ; Bridgeford, Qu'Appelle Valley B. de Vries 846 (DAO 281697) ; Davidson E.W. Sullivan 87 (REG s.n.) ; Davidson G.F. Ledingham et al. 2277 (REG s.n.) ; Qu'Appelle Valley, 20mi N of Regina G.F. Ledingham et al. 5439 (REG s.n.) ; Buffalo Pound Lake G.H. Turner s.n. (ALTA 65977) ; Lawson, Lake Centre District I.J. Bassett & K.F. Best 4096 (DAO 281693) ; Qu'Appelle River Dam J. & F. Pigott s.n. (WIN 37894) ; Elbow (10 mi S) J. Looman 17137 (SHER 148672, DAO 167118) ; Bethune-Davidson J.B. Campbell 2580 (UAC 26728) ; Dundurn J.B. Gollop (SASK 71105, REG s.n.) ; Govan J.F. Alex & J.P. Gebhardt 2598 (DAS 280204) ; Guernsey J.F. Alex & J.P. Gebhardt 2610 (DAS 280200) ; Regina J.F. Alex & J.P. Gebhardt 2659 (DAS 280202) ; Mortlack, (NE), Moose Jaw J.H. Hudson 1668 (DAO 281699) ; Homefield J.P. Gebhardt 62 (DAS 280203) ; Renown R.D Bibbey s.n. (SASK 22938) ; Girvin R.T. Coupland 247 (SASK 22935, DAO 281702) ; Bladworth S. Zilke 41 (SASK 22933, DAO 39032, SASK 22931) ; West of Saskatoon W.A. Johnstone s.n. (DAO 281688) ; Wilkie, Battleford District W.D. Garratt s.n. (SASK 22932)

BC. : Airport at Princeton J. Grant 31 (DAO 384036).

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