UNIVERSITY OF SASKATCHEWAN

Medicinal and Aromatic Plant Research

Objective 1. Introduction and evaluation of new/improved lines of cumin and milk thistle

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Saskatchewan Agriculture and Food

Medicinal and Aromatic Plant Research
Program Background

Production and processing of spice crops and medicinal herbs represents a potential bright spot in the future of Saskatchewan's agricultural sector. Saskatchewan growers have established a worldwide reputation as cost-competitive suppliers of superior quality medicinal plants and spices. Based on present growth rates, the Saskatchewan Herb and Spice Association (SHSA) projects the value of primary production of Saskatchewan-grown herb and spice products will approach $ 200 million annually by 2010. Numerous opportunities are available to add further value to these crops through processing, blending, preservation, extraction, encapsulation and packaging. A number of Saskatchewan companies have seized these opportunities, resulting in significant employment and economic activity in both urban and small town settings.

The marketplace for medicinals and spices is demanding, rapidly changing and highly competitive. To stay viable, Saskatchewan's herb and spice sector must focus on the best crops, varieties and production practices available. Emerging threats such as disease must be identified and dealt with effectively, but in a manner that does not jeopardise Saskatchewan's reputation for producing a safe, quality product.

This project takes a multi-disciplined approach to address the key production challenges for cumin and milk thistle under the relatively short and cool growing conditions in Saskatchewan. Production of these crops in Saskatchewan is presently limited by challenging growing conditions and specific agronomic problems. This project aims to alleviate these problems by crop improvement and development of superior agronomic practices.

Although improvement of spice/medicinal crops is possible utilizing standard plant breeding methods - the process is slow and consequently expensive. Double haploid technology allows the creation of a genetically homogeneous population without the need for multiple generations of selfing. This has the potential to greatly accelerate progress in improvement of medicinal/aromatic plants.

Program Objectives

1) Introduction/development of new/improved lines of cumin and milk thistle by:

a) accessing and evaluating potentially suitable material from public and private sources.
b) working with breeders from the Crop Development Center of the University of Saskatchewan to further develop adapted crop lines.

2) Pathology support to reduce losses to disease in cumin:

Blossom blight has decimated previous plantings of cumin in Saskatchewan.
a) Agronomically superior lines of cumin identified under Objective #1 will be evaluated for disease sensitivity in a disease nursery previously established by the Dept of Plant Sciences.
b) An integrated disease management approach will be developed, involving selection of resistant lines, identification of preventative production practices and evaluation of chemical control options.

3) Agronomy of Milk Thistle:

a) Time of seeding, seeding rate and row spacing effects on growth, yield and quality characteristics.
b) Optimizing nitrogen and phosphorus application rates.
c) Comparing organic products to standard chemicals for desiccation of the crop.

4) Field performance of new lines of spice/medicinal crops created using double haploid technology:

a) To evaluate lines of dill, fennel, anise, and cowcockle created by PBI/NRC using double haploid technology.
b) to compare the performance of the double haploid lines to parental lines

Objective 1. Introduction and evaluation of new/improved lines of cumin and milk thistle.

At present, growers attempting to produce cumin in Saskatchewan typically obtain seed from Syria and/or Turkey which are also the dominant suppliers of this commodity at this time. The material obtained so far from these sources has been too short of stature to be mechanically combined and too late to achieve consistent yields under the relatively short Saskatchewan growing season. Blossom blights have also been problematic in the limited number of cumin lines that have been tested in Saskatchewan.

Although milk thistle has been used medicinally for centuries, it has only been under cultivated production for a few decades. Most of the milk thistle planting material presently available to growers was derived by selection and multiplication of plants from wild stands. Efforts to enhance agronomic perform or quality of milk thistle through systematic breeding have been limited. Consequently the crop has considerable potential for improvement in terms of yields, growth habit, quality (as indicated by silymarin content), uniformity of maturity and particularly in its resistance to shattering.

Screening trials represent a highly cost effective means for identifying material adapted to specific environments, production methods or markets. The project accessed promising lines of both milk thistle and cumin from local sources and areas where these crops have a longer history of production (India, the Middle East and Eastern Europe for cumin and Central Europe for milk thistle). The Plant Gene Resource Centre (PGRC) of Agriculture Canada located in Saskatoon (Dr. A Diederichsen B Curator) assisted by accessing worldwide germplasm collections of these crops

This project evaluated all available lines of cumin and milk thistle under typical Saskatchewan production conditions B looking for promising lines or parental material for future improvement programs.

Cumin Germplasm Trials

Cumin Germplasm Trials in 2003

Samples of 18 cumin lines from 6 different countries were field tested in 2003. The 2003 test was conducted at the Department of Plant Sciences, Horticultural Crops Research Station in Saskatoon. This site features a relatively heavy Sutherland Series clay soil which is not ideal for planting or emergence of small seeded crops. The site is however convenient, sheltered and has access to irrigation - factors regarded as useful when conducting screening trials.

The trial site was prepared by disking, then rotovating two weeks prior to planting. Fertility levels in the test area were adjusted to 100 kg N/ha and 80 kg P2O5/ha. which correspond with recommendations for other spice crops. The site was lightly irrigated a week prior to planting. Seeding was delayed until the 2^{nd} week of June to allow the soil to warm to temperatures favorable for the rapid germination of cumin. Where sufficient seed was available, the plots were seeded with a press drill type small plot seeder. This unit seeds 6 rows at a time, with the rows spaced 15 cm apart with 3 cm between seeds in a row. The test plots were 6m long. At least two replicate plots of each line were planted. In a few cases, insufficient seed was received to allow for direct seeding of a line. This material was started in the greenhouse in early May and transplanted into the field in early June.

Although care was taken to insure that the seed was placed into a well prepared seedbed under near-ideal conditions for germination, emergence percentages were poor. The most vigorous line produced less than 30% stand. Part of this problem can be attributed to problems with seed quality. Even under the near ideal conditions in the greenhouse (20/18 ^{o}C day/night), some lines failed to produce a single plant. However, problems with the seeder and/or the seedbed preparation also contributed to the poor stand. This was indicated by the fact that within each plot emergence % were non-uniform. Some drill rows showed excellent emergence ... while other rows did not produce a single plant. Subtle variations in the depth of planting between drill rows may have been influencing emergence %.

The dominant weed in the test area was common groundsel *(Senecio vulgaris*). Although groundsel is a slow germinating and non-aggressive plant, it has the potential to exert significant competitive pressure on slow growing, short stature crops like cumin. No herbicides were applied in this trial in order to avoid confusing agronomic performance with issues of herbicide sensitivity. Weeds were controlled by hand. Several weeding operations were required due to the poor stand and slow growth of the cumin crop.

Problems with disease were evident from emergence of the crop through until the final harvest. In cases where the crop was in the greenhouse, some lines showed significant amounts of disease before the plants were old enough to be transplanted into the field. The fact that other lines were apparently healthy suggest ... a) genetic differences in sensitivity to disease or b) non-uniform exposure to disease inoculum (ie; seed contamination ?). Once moved into the field, many of the transplants quickly succumbed to disease. The fact that adjacent transplanted and direct seeded lines stayed healthy would seem to again indicate a) genetic differences in sensitivity to disease or b) non-uniform exposure to disease inoculum (ie; seed contamination ?). Symptoms of the disease involved ... rapid discoloration of the leaves, followed by chlorosis then necrosis. The problem usually began with the older leaves but encompassed the entire seedling within 48 hours. The problem seemed to associated with early crop growth stages or conditions encountered early in the growing season ... as no new problems with disease were observed after mid-July. We isolated a number of potentially pathogenic species from the infected leaves *(Fusarium, Ascochyta, Alternaria*) but were unable to determine which (if any) was the primary pathogen.

The plot area was irrigated occasionally from late July onwards as drought conditions were severe. No significant insect problems were observed despite that fact that the test area experienced a severe grasshopper infestation.

The plots were harvested by hand in early October. The plants were cut at ground level and placed into cloth bags which were stored in a 60C drier for 5 days. The seed was then threshed, cleaned and weighed.
The germination % of each original seed lot was evaluated using a standard filter paper test. Stands were visually evaluated in mid-season, after hand removal of weeds. The germination % of each seed lot resulting from the 2003 cropping season were also evaluated after 4 months of cold storage in January of 2004.

Table 1.1. Agronomic performance of Cumin lines tested in 2003.

Line	Country of Origin	Germ (1) (%)	Stand (%)	Yield (kg/ha)	Corrected Yield (kg/ha)	Germ (2) %
Austra Hort	Holland	99	50	507	912	4
Vesey's	(?)	63	40	298	670	4
Cruz	India	68	20	104	468	0
William Dam	(?)	94	60	402	602	12
Johnny's	(?)	65	50	331	596	7
Kalyx 1	India	76	15	6	36	1
Kalyx 2	Egypt	48	60	666	1000	3
Richters	?	96	35	59	151	3
Royston	Holland	8	10	140	1260	6
Bolier	Holland	85	50	248	446	3
Eden Seeds	Holland	85	-	-		-
TR 56069	Turkey	39	100 (T)	1441	1441	14
TR 56071	Turkey	30	100 (T)	642	642	23
TR 56072	Turkey	31	100 (T)	1003	1003	20
TMP 13729	Afghanistan	0	0	0	0	-
TMP 13728	(?)	56	100 (T)	76	76	61
U of S	CDN	0	0	0	0	-
Diefenbaker Seed DSPYG.5Y	(?)	61	50	260	468	18
Diefenbaker Seed DSPYG.1N	(?)	70	25	69	248	6

? = unknown or not specified.
Germ 1 = germination % of original seed lot.
Germ 2 = germination % of the seed originating from the 2003 crop grown in Saskatoon
Corrected yield = yield corrected to approximate results obtained with a complete stand.

Germination of Original Seed Lots - even under the near ideal conditions provided in the germination tests, the average germination % was only 56 %. Some lines germinated well while others appeared either dead or dormant.

Stand % - emergence of the direct seeded lines was relatively slow and uneven. The small seeded cumin appeared ill equipped to deal with the relatively heavy soil at the test site. There was significant variability between and within replicates for % stand ... this suggests problems with both field conditions and seed performance. All the transplanted lines survived the transplanting step to produce a virtually perfect stand.

Yields - yields of the direct seeded lines were generally poor, averaging just 240 kg/ha (0 to 666 kg/ha). Although the obviously immature seeds were removed in the grading process, the overall maturity levels of the 2003 crop were not good. This is reflected by the extremely low germination % obtained when the "marketable" seeds from the 2003 crop were tested after 4 months cold storage. Much of the seed harvested in 2003 lacked the visual characteristics (large and plump) and flavor required by the culinary trade. Transplanting allowed achievement of a near perfect stand and also provided an additional 3 weeks of growing time for these lines. It is therefore not surprising that the highest yield (1440 kg/ha) was obtained from a transplanted line. Transplanting did not overcome all problems experienced by the cumin crop - some of the transplanted lines developed foliar disease soon after transplanting. This disease reduced the vigor of the affected plants but did not appear to spread from plant to plant or into the direct seeded lines. Transplanting also failed to overcome problems with crop maturity - the visual appearance of the seed obtained from the lines transplanted in 2003 was little better than the direct seeded lines.

Yields Corrected for Stand - to allow for effective comparisons of the relative yield potential of the direct seeded versus the transplanted lines - we corrected yields of the direct seeded lines to approximate those that could have been anticipated if a 90% stand had been achieved. These calculations assume;

a) that a 90% stand is achievable
b) that no yield compensation occurred as a function of the limited stand produced in this trial

Assumption a - seems reasonable if high quality seed was planted out at an appropriate rate into a well-prepared field featuring a suitable type soil.
Assumption b - also seems reasonable as the cumin plants failed to completely utilize the available growing spacing even in the transplanted plots where a complete stand was achieved.
Based on these assumptions ... the corrected yields of some of the direct seeded lines did approximate those obtained when a complete stand was achieved by using transplants. Yields approximating 1t/ha represent the norm for cumin production in India, but are far below grower expectations in Canada.
Of the direct seeded lines, Austra Hort and Kaylx 2 appeared to combine reasonable crop vigor and early yields. Of the transplanted lines, TR56069 and TR56072 had the highest yields and vigor.

Germination of Seed Generated in 2003 - the seed produced in 2003 had an exceptionally low average germination %. This suggests that the material harvested in 2003 was still physiologically immature, despite the fact that the growing season was generally quite favorable. Use of the crop covers as a means of maintaining the genetic purity of the lines had little impact (+/-) on the germination potential of the seed.
The poor germination performance of the seed produced in 2003 necessitated the acquisition of new material from the original seed sources for planting in 2004.

Cumin Germplasm Trials in 2004

Methodology

Because problems with stand establishment, root rot and control of certain weeds were observed on the clay soil used in 2003, the 2004 trial was shifted to a site with a lighter textured sandy loam soil (University of Saskatchewan Potato Research plots - Saskatoon). This site was planted to irrigated potatoes in 2003 and had a very low weed pressure.

The trial site was prepared by disking, then rotovating two weeks prior to planting. Residual fertility levels in the test area were adequate to meet the needs of the cumin crop. The day prior to seedling, the plot area was treated with the herbicide ethafluralin (Edge). The trial was seeded on May 14 which is far earlier than in the 2003 trial. This earlier seeding date corresponded with recommendations by commercial growers. A precision small plot seeder was used to seed the trial. Each line was seeded in a 4m long plot with 4 rows/plot. The rows were spaced 30 cm apart with 10 cm between plants within the row. The planting was replicated twice in a randomized complete block design.

Although considerable care was taken to insure that the seed was placed into a well prepared seedbed under near-ideal conditions for germination, emergence percentages were spotty. Some lines produced no plants, while others produced almost a perfect stand. Similar problems with stand establishment were observed in the 2003 trial. Part of this problem can be attributed to problems with seed quality. In benchtop germination tests some lines failed to produce a single plant.

Weed pressure was light in this trial. Any escapes from the herbicide program were removed by hand. With the onset of the hand weeding program in late June (3 weeks after emergence) it was observed that the stand had further deteriorated in many lines. The affected seedlings appeared to be drying up despite the presence of adequate soil moisture. Adjacent plants in other rows or lines also appeared to be free of drought stress. The root systems of the affected plants were small, discolored with poor lateral root development. Losses to this problem continued through mid-July, by which time the only line with more than a handful of survivors was from William Dam Seeds.

Similar problems with loss of plants beginning at emergence and continuing for the duration of the season were observed... a) in the 2003 trials, b) in research plots in adjacent field areas in 2004 and c) in a commercial field in 2004. The symptomology is suggestive of root rot which can be caused by a range of seed and soil-borne organisms. A range of organisms, including Fusarium and Pythium were isolated from the root systems of the affected plants. Both of these organisms have the potential to cause root decay leading to wilting type disease.

The plot area was irrigated occasionally from late July onwards. Although weather conditions at flowering were conducive to development of blossom blight, none was observed. Development and spread of this disease problem may have been limited by the sparse plant populations.

The plots were harvested by hand on September 7, following the first killing frost. The plants were cut at ground level and placed into cloth bags which were stored in a 40C drier for 5 days. The seed was then threshed, cleaned and weighed.

Table 1.2. Agronomic performance of Cumin lines tested in 2004.

Line	Country of Origin	Stand (%)	Yield (kg/ha)	1000 seed weight (g)
Austra Hort	Holland	50	17	2
Vesey's	(?)	25	16	5.5
Cruz	India	10	0	2.5
William Dam	(?)	65	90	4.1
Johnny's	(?)	20	4	5.6
Kalyx 1	India	10	2	3.7
Kalyx 2	Egypt	15	6	3.2
Richters	?	35	0	1.1
Royston	Holland	10	20	3.4
Bolier	Holland	0	0	-
Eden Seeds	Holland	0	0	-
U of S	CDN	0	0	-
Diefenbaker Seed DSPYG.5Y	(?)	0	0	-
Diefenbaker Seed DSPYG.1N	(?)	0	0	-

? = unknown or not specified.

Yields were exceptionally poor, largely as a function of the poor stand and plant losses over the duration of the season. Quality of any seed that was harvested was also poor. In many of the lines, the seed was shrunken and appeared to be immature despite the fact that the crop had ample time to mature. This suggests that the plants have been unable to support normal seed development. Only the William Dam line produced any quantities of seed that would have met commercial grade standards for size and color

The poor germination performance of the seed produced in 2003 coupled with the general crop failure in 2004 necessitated the acquisition of new material for planting in 2005.

Milk Thistle Germplasm Trials

Milk Thistle Germplasm Trials in 2003

Samples of 24 lines of milk thistle from 8 different countries (Table 1.3) were tested in 2003.

The field test was conducted at the Department of Plant Sciences, Potato Research Plots in Saskatoon. This site features a relatively light sandy loam soil which is ideal for cropping if it can be irrigated. The site lacks shelter but has access to overhead irrigation.

The trial site was prepared by disking, two weeks prior to planting. Fertility levels in the test area were adjusted to 90 kg N/ha and 80 kg P2O5/ha. which correspond with recommendations for herbaceous medicinals. Seeding was delayed until early June to allow the soil to warm to temperatures favorable for the rapid germination of the milk thistle seed and to avoid frost damage to the sensitive seedlings. Where sufficient seed was available, the plots were seeded by hand in rows spaced 50 cm apart with 30 cm between seeds in a row. The total area of each test plot was $9m^2$. At least two replicate plots of each line were planted. In a few cases, insufficient seed was received to allow for direct seeding of a line. This material was started in the greenhouse in early May and transplanted into the field in early June.

Although considerable care was taken to insure that the seed was placed into a well prepared seedbed under near-ideal conditions for germination, emergence percentages of some lines were extremely poor (Table 2). Conversely other lines produced excellent stands - this suggests that the problem stands reflects problems with the seedlot rather than the field conditions. Even under the near ideal conditions in the greenhouse (20/18 ^{o}C day/night), some of the milk thistle lines failed to produce a single plant (Table 2).

If the initial flush of pigweed was controlled, the milk thistle was capable of out-competing most weeds. No herbicides were applied in this trial in order to avoid confusing agronomic performance with issues of herbicide sensitivity.

Although growers have reported problems with Aster Yellows in Milk thistle, no problems with disease were observed in this trial. Richardson’s Ground Squirrels will eat young milk thistle plants.

The plot area was irrigated occasionally from late July onwards as drought conditions were severe. By August a severe grasshopper infestation was evident ... but the milk thistle was obviously not a favored food species.

Table 1.3. Milk thistle lines tested in 2003.

Line	Country of Origin	Germ (%)	Stand (%)	Vigor at Harvest (0-5)	% heads matured	Yield (g/m2)	Yield (kg/ha)
Austra Hort	Holland	-	100	5	80	5.6	56
Bolier	Holland	-	84	1.7	50	7.8	78
Cruz	Eastern Europe	-	0	-	-	0	0
Genesis	Israel	-	100	2.5	25	3.6	36
Horizon Herbs	?	-	100	5	< 10	0.4	4
Johnny's	?	-	72	4	< 10	0	0
Kalyx 1	Holland	-	66	3.2	80	3.8	38
Kalyx 2	Bulgaria	-	0	-	-	0	0
Richters	?	-	74	2.5	70	3	3
Royston Lot 1236 LJ2	Germany	-	46	3	variable	0	0
Royston Lot 757PJ2	Germany	-	42	2.7	50	0.5	5
Secret Seeds	Afghanistan (?)	-	54	1	< 10	0	0
TMP 13730	(?)	81	90	1.5	80	18.1	181
TMP 13868	USSR	43	53	0	65	9	90
TMP 13920	Czech	100	22	0.5	50	8.9	89
TMP 13921	Czech	95	4	0.5	< 10	0	0
TMP 13922	Czech	100	77	1	50	18.1	181
TMP 13923	Czech	90	81	4	80	4.3	43
TMP 13924	Czech	95	43	0	50	27.3	273
TMP 13925	Czech	100	30	1	70	21.4	214
TMP 13926	Czech	100	13	0	50	8.6	86

trnplt = transplanted.

The plots were harvested in early October after several killing frosts. Seed heads were harvested by hand and then air dried at 40 C for 5 d. The seed heads were threshed and the seeds cleaned, sorted for maturity and weighed.

Analysis of the crop vigor and head maturity data at harvest suggested that some milk thistle lines were far better suited to SK growing conditions than others (Table 1.3). In some of the lines the plants had died back and all virtually all the heads were mature prior to harvest which occurred soon after the first killing frost. By contrast, other lines were growing vigorously at harvest and had few if any flowers that had reached the harvestable stage by the end of the growing season.

When seed yields are considered;

a) good stand % contributed to good yields, but were not a guarantee of good yields.
b) there was a negative relationship between plant vigor and seed yield.

This was particularly apparent when comparing the direct seed versus transplanted lines. The transplanted lines appeared to lack vigor throughout the growing season, yet they substantially out-yielded the direct seed lines. The highest yielding line (TMP 13924) actually had the lowest plant vigor rating at harvest of all the lines evaluated. This suggests that this line devotes a disproportionately large amount of its total productivity to early seed production. This may actually be advantageous as the rank growth habit of milk thistle makes it extremely difficult to harvest. Line TMP 13924 would have been much higher yielding if it had produced a reasonably complete stand. Yields of even the best lines in this trial were far below the levels needed for milk thistle to represent an economically viable crop in Saskatchewan (ca 500 kg/ha).

Quality analysis

"Silymarin" content is the commonly applied indicator of milk thistle quality. Silymarin content is actually the sum of Silybinin A + Silybinin B + other flavonolignans (dehydrosilybinin, silychristine, silydianine and taxifolin). Each of these molecules has its own form of therapeutic activity and value and therefore should be reported separately. Milk thistle with a silymarin content of 1.5-3.0 % is considered "good quality" by the industry, while Saskatchewan milk thistle typically ranges from 0.8-3.0 % silymarin (W. Wolf 2004 - pers comm).

Seed samples from the 2003 crop were analyzed for their quality components using extraction and analysis techniques developed by Dr. Branka Barl of the Herb Research Center. The key steps in the analysis were;

1) 5 g samples of dried clean seed were ground and passed through a 24-mesh (710 um) sieve.
2) 1 g of the sieved material was wrapped in filter paper and then extracted with 100 mL of petroleum ether for 4 h in a Soxhlet extractor. This step removes the fixed oils.
3) The sample package was dried overnight and then re-extracted for 4 h using 100 mL methanol. This extracts the silymarin complex.
4) a 1 mL aliquot of the methanol solution was filtered through a 45 um syringe.
5) the cleaned sample was analyzed using HPLC

The quality of the milk thistle lines evaluated in 2003 was quite variable - both in terms of total silymarin content and the component profile (Table 1.4). The average total silymarin content (1.6%) was at the low end of the acceptable quality range - however some lines appeared to have excellent quality (ie; Richters, Royston, TMP 13925 and TMP 13926).

It is interesting to note that quality characteristics of a milk thistle line planted in the fall (Richter's) was very comparable to the same line planted in the spring. Similarly, the quality profile of milk thistle
(cv. Richter's) exposed to a range of fertility treatments was also quite consistent. This suggests a degree of quality stability despite differing growing conditions and production practices.

Table 1.4 Quality characteristics for Milk Thistle lines evaluated in 2003

Line	Silibinin A (% w/w)	Silibinin B (% w/w)	Other Flavonolignans	Silymarin (% w/w)
Austra Hort	0.29	0.38	0.81	1.5
Bolier	0.33	0.45	0.93	1.43
Genesis	0.06	0.07	0.94	1.06
Kalyx 1	0.31	0.44	0.92	1.67
Richters	0.52	0.72	1.07	2.35
Royston Lot 757PJ2	0.49	0.69	1.21	2.39
TMP 13730	0.06	0.07	0.88	1
TMP 13868	0.21	0.27	1	1.47
TMP 13920	0.04	0.05	1.02	1.1
TMP 13922	0.06	0.07	0.9	1.03
TMP 13923	0.06	0.07	0.9	1.01
TMP 13924	0.07	0.08	1.07	1.21
TMP 13925	0.44	0.62	1.03	2.1
TMP 13926	0.69	1.03	1.49	3.22
Mean	**0.25**	**0.36**	**0.92**	**1.61**
Richters - Fall planted	0.39	0.6	1.26	2.26
Fertility trials (Richter's)				
0 N + 0 P2O5	0.35	0.49	1.17	2
0 N + 120 P2O5	0.37	0.53	1.26	2.16
100 N + 0 P2O5	0.36	0.51	1.27	2.14
100 N + 120 P2O5	0.39	0.56	1.37	2.32

Milk Thistle Germplasm Trials in 2004

Lines tested in 2004 were; a) lines tested in 2003 and b) any additional lines received.

The field test was conducted at the Department of Plant Sciences, Horticulture Field Research Station in Saskatoon. This site was selected to overcome some of the problems observed in the 2003 trial - specifically the 2004 site has no gopher problems. This site also features a much heavier, clay soil than was used in the 2003 trials which would allow production of the crop with less frequent irrigation. The 2004 site is well protected by a shelterbelt system.

The trial site was prepared by disking, then rotovating two weeks prior to seeding. As a function of heavy fertilizer applications in previous crops, fertility levels in the test area were > 100 kg N/ha and > 120 kg P2O5/ha. These fertility levels would easily meet the needs of most herbaceous medicinals and may actually be somewhat excessive - potentially leading to excessive vegetative growth. Seeding occurred earlier than in 2003 (May 17) in an attempt to maximize the length of the growing season. Where sufficient seed was available, the plots were seeded using a push-type precision small plot seeder. Rows were spaced 0.75 m apart with 0.75 m between plants within the row. There were three rows in each plot giving a total plot area of $12m^2$. There were two replicates arranged in a randomized complete block design. In a few cases, there was insufficient seed to allow for direct seeding. In those cases, the plants were started in the greenhouse in early May and transplanted into the field on May 20.

Emergence percentages for most lines were very high and some lines had to be hand thinned to prevent overcrowding. In some lines (Reddis and Cruz E) however emerge was poor despite obvious favorable germination conditions. This suggests that the poor stands reflects problems with the seedlot rather than the field conditions - particularly as these lines also had emergence problems the previous year.
As milk thistle is slow to establish, some weed control was needed. However, the wide row and plant spacings makes tillage simple. No problems with weed competition occurred in 2004 - although there was obviously competition between milk thistle plants within the row. No herbicides were applied in this trial in order to avoid confusing agronomic performance with issues of herbicide sensitivity.

Although growers have reported problems with Aster Yellows in Milk thistle, no problems with disease were observed in this trial. No insect or other pest problems were observed.

The plot area was irrigated occasionally - but not as frequently as in 2003 as the 2004 growing season was cool and wet and the clay soil at the site had a high water holding capacity. The crop was not watered after the start of September in an effort to promote maturation.

The plots were not damaged by a light frost that occurred in mid-August. The plots were harvested by October 12 after one killing frost and several subsequent lighter frosts. After these frost events the stalks were quite dry but shattering was minimal in most lines. Crop maturity was visually rated just prior to the harvest. Plant heights were also determined at that time. One of the reps was harvested by hand while the other rep was harvested using a Wintersteiger Nursery Master Elite small plot combine. The combine was adjusted to minimize seed losses by reducing the fan speed and opening the sieves. The harvested material was air dried at 40 C for 5 d, threshed and then cleaned using a dockage sorter. Seed yields and 1000 seed weights were determined at this point. For the quality analysis a seed sample was further cleaned by hand to eliminate all impurities. Yields from the mechanically harvested replicates were 80% of those obtained by careful hand harvest. Although this represents a substantial combining loss, the savings in labor far exceed the value of the material lost. Hand harvest of milk thistle is slow, painful and unless done carefully can actually increase losses due to shattering.

The stand, maturity index and yield data suggest that some milk thistle lines were far better suited to SK growing conditions than others (Table 1.5). As the 2004 growing season was relatively cool, the crop was generally not as advanced by harvest time as in 2003. There were however some lines that were quite mature by harvest in 2004 (Bolier and Royston Lot 131), while other lines (Secret Seeds) were just beginning to flower by harvest time

When seed yields are considered;

a) good stand % contributed to good yields, but was not a guarantee of good yields.
b) several of the lines had yields that exceeded 1000 kg/ha. This is promising as the yield levels needed for milk thistle to represent an economically viable crop in Saskatchewan are ca 500 kg/ha. It is important to note that yields in small plot field trials are a relatively poor indicator of the yields obtained under the less intensively managed commercial production conditions.
c) there was little correlation between seed size and yield

Qaulity Analysis

The quality data for the various milk thistle lines tested are presented in Table 1.6, along with the quality data for selected treatments from the agronomy trials.

Some key quality observations from the germplasm evaluation trials are;

a) quality of the 2004 crop was inferior to the 2003 crop. The average silymarin content in 2004 was 1.4% as compared to 1.6% in 2003. The relatively cool weather in 2004 may have delayed crop maturity, resulting in a corresponding decline in seed quality. There were however a number of lines that produced acceptable quality (>2% silymarin) in 2004.

b) relative seed quality was not consistent across years ... some of the cultivars with the highest silymarin content in 2003, performed poorly in 2004 and visa-versa.

c) the method of harvest had little impact on seed quality ... in fact the quality of the machine harvested crop was slightly higher than for the hand harvested crop. This quality differential was unexpected ... as it was assumed that the non-selective nature of the machine harvest would result in a lower grade product. Seed yields were lower in the machine harvested plots ... but the quality data suggests that the seed that was lost during machine harvest was of poor quality - likely immature.

d) total silymarin yield (seed yield * silymarin content) is the ultimate measurement of a cultivar's suitability ... assuming that silymarin content is a factor in sale price.   Austra Hort and Bolier were outstanding for total silymarin yield in the 2004 trial. These cultivars combined both excellent seed yields and high seed quality. It is interesting to note the strong positive correlation between seed yield and seed quality in the 2004 trial.

Table 1.5. Growth and yield characteristics for Milk Thistle lines in 2004.

Line	Country of Origin	Stand (%)	Plant height (cm)	% heads matured	Yield (kg/ha)	1000 seed wt (g)
Austra Hort	Holland	84	140	75	1337	22.4
Bolier	Holland	94	160	75	1254	23.7
Cruz	Eastern Europe	<5	-	-	-	23.8
Genesis	Israel	96	150	72	823	27.4
Horizon Herbs	?	100	200	65	656	17.8
Johnny's	?	100	180	60	643	18.5
Kalyx 1	Holland	94	180	50	1151	25.5
Kalyx 2	Bulgaria	0	-	-	-	-
Richters	?	86	200	50	870	20.2
Royston Lot 1236 LJ2	Germany	T	180	45	1007	18.1
Royston Lot 757PJ2	Germany	T	180	75	-	-
Secret Seeds	Afghanistan (?)	100	60	< 10	0	-
TMP 14068	(?)	T	180	70	1362	23.2

Seeding density and between row spacings had relatively little impact on seed quality of milk thistle (cv. Richter's) in 2004 (Table 1.6). It was hypothesized that a higher plant population, would reduced the plants' tendency to remain vegetative ... resulting in more uniform flowering and higher seed quality.

The time and method of top-kill also had no consistent effect on quality of the milk thistle seed (cv. Richter's) in 2004 (Table 1.6). It was expected that delaying top-kill until the crop was more mature (60% bloom) would increase seed quality.

Table 1.6 Quality characteristics for Milk Thistle lines evaluated in 2004

Line	Silibinin A (% w/w)		Silibinin B (% w/w)		Other Flavonolignans		Silymarin (% w/w)		Silymarin yield (kg/ha)
	Hand	**Combine**	**Hand**	**Combine**	**Hand**	**Combine**	**Hand**	**Combine**	
Austra Hort	0.2	0.38	0.27	0.53	0.85	1.13	1.32	2	22.2
Bolier	0.38	0.37	0.53	0.52	1.03	1.29	1.94	2.18	25.8
Cruz	-	0.41	-	0.58	-	1.1	-	2.09	-
Genesis	0.1	0.05	0.1	0.06	0.56	0.85	0.64	0.95	6.5
Horizon	0	0.04	0.1	0.05	0.46	0.71	0.55	0.81	4.5
Johnny's	0.1	0.04	0.1	0.05	0.74	0.54	0.93	0.62	5
Kalyx 1	0.27	0.37	0.38	0.52	0.89	1.18	1.52	2.07	20.8
Reddis	0.31	0.31	0.37	0.44	0.75	0.78	1.42	1.53	-
Richters	0.32	0.42	0.39	0.58	0.99	1.2	1.7	2.2	16.9
Royston 757PJ2	0.52	-	0.74	-	1.29	-	2.54	-	-
Royston 1236LJ-2	0.1	0.12	0.1	0.15	0.6	0.78	0.79	1.06	9.3
Royston 131-J2	0.3	0.18	0.35	0.21	0.79	0.64	1.44	1.02	-
TMP 14068	0	0.05	0.1	0.05	0.64	0.61	0.73	0.71	9.8
Mean	**0.2**	**0.23**	**0.28**	**0.31**	**0.8**	**0.9**	**1.29**	**1.43**	**13.4**

Spacing trials ... Richter's @ 25 or 150 seeds/m2 with 20 or 60 cm between rows					
rate = 25 * 20 cm	0.26	0.31	0.96	1.53	
rate = 25 * 60 cm	0.19	0.23	0.87	1.29	
rate = 150 * 20 cm	0.24	0.28	0.94	1.46	
rate = 150 * 60 cm	0.29	0.35	1.13	1.77	
Top Kill Trial .... Reglone or Vinegar @ 30% or 60% bloom					
Vinegar @ 30% bloom	0.23	0.27	0.9	1.39	
Vinegar @ 60% bloom	0.21	0.26	0.93	1.4	
Reglone @ 30% bloom	0.25	0.3	0.95	1.5	
Reglone @ 60% bloom	0.23	0.27	0.93	1.42	

Grading to Improve Milk Thistle Quality

It has been established that the quality of milk thistle increases as the seed matures. Therefore the quality of a given seed lot can be improved if the seed can be sorted for maturity. Immature milk thistle seeds are small, thin and quite light in color by comparison with mature seed. Milk thistle seed cab be easily sorted with readily available equipment based on size and density. This does an acceptable job of sorting out the very immature seeds ... but cannot achieve any further grade improvement. It is possible however, to further sort based on seed color. In 2003, we used a color sorter to grade a seed lot (Richter's) and then examined the silymarin content of the resulting grade categories. There was some tendency for the silymarin content to increase with increasingly dark seed color. In 2004, we ranked the various cultivars tested in the germplasm evaluation trial for seed coat color (darkest = rank of 1) and silymarin content (highest = rank of 1)(Figure 1.7). We found that there was little direct association between seed coat color and silymarin content. We also ranked various lots of the cv. Richter's grown in differing trials (* in Fig. 1.7). Again, there is little in the way of a relationship between coat color and silymarin content. This suggests limited potential to use color sorting technology to further improve sorting for quality in milk thistle.

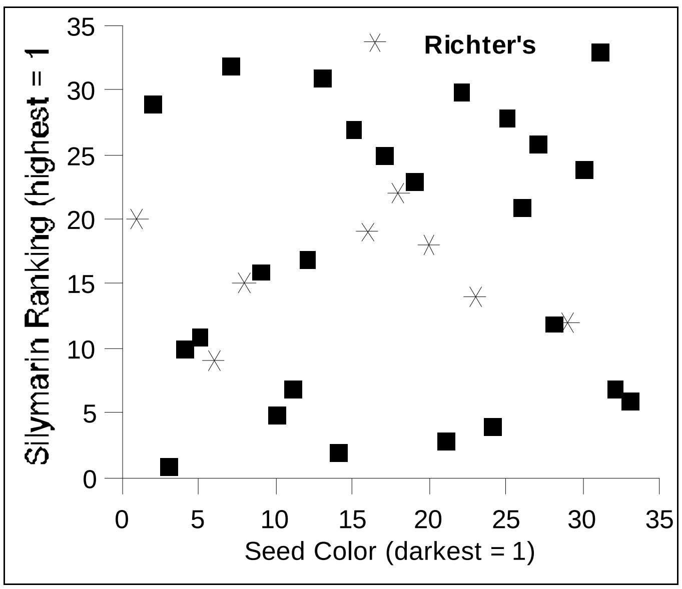

Figure 1.7