Preface to the following paper. This paper is based on the text of a talk given by R. P. Murphy, January 22, 1976, at a Plant Breeding workshop sponsored by the Department of Plant Breeding and Biometry of the New York State College of Agriculture and Life Sciences at Cornell, and the National Council of Commercial Plant Breeders. Dr. Murphy agreed to let us include this paper in the TAG proceedings. The paper contains important information about the use of germplasm in the development of the alfalfa genome.

THE USE OF EXOTIC GERMPLASM IN ALFALFA BREEDING

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Introduction

Alfalfa is a perennial, cross-pollinated, autotetraploid forage legume which is a premier forage crop in the temperate and dry sub-tropical zones of the world. The primary center of origin is thought to be the Near East and Central Asia. Related species and genera are widely distributed in these areas. Although alfalfa is an important crop in its center of origin, its greatest use is in other areas of the world. Forty percent of the world=s acreage is in North America, 24% in South America and 28% in Europe including European Russia. Over 58% is in two nations - the United States and Argentina. It is probable that much of this vast acreage of alfalfa, 33 million hectares (80 million acres), is planted to sorts represented by only a small amount of the germplasm that exists in the center(s) of origin.

As are all crops, alfalfa is to some degree vulnerable because of a limited germplasm base. This may be due to the lack of a specific gene or genes that control the reaction to a disease or pest or to a lack of gene diversity that influence vigor, adaptation and other quantitative characters. In addition as agriculture in the center of origin becomes more specialized, existing diverse sorts may be lost. Currently many seed collections exist but they are not being used to any great degree nor is there any coordinated effort to change this situation. Only specific introductions or specific characteristics have been used. The U.S. collections are primarily from the Near East. According to Skrdla, North Central Regional Plant Introduction Station, Ames, Iowa, a collection of 902 introductions are maintained. There are relatively few from Central Asia and other areas in the USSR and China. The USSR presumably has an extensive internal collection. The condition of the seed collections in both instances is not well known and meaningful evaluations of their value to breeders is generally lacking. Where introductions have made useful contributions to cultivar improvement it has been usually by the use of specific characters such as bacterial wild resistance and winter hardiness. An exception to this has been the contribution of Medicago falcata, both diploid and tetraploid sorts, to vigor, winterhardiness and adaptation in the humid temperate zones of North America

and Northern and Central Europe. This has often been by chance as much as by design but many of the important cultivars in these areas have variegated flowers indicating a significant introgression of falcata germplasm into sativa, the purple-flowered alfalfa. Another exception was the development of Narragansett and Atlantic cultivars in which large numbers of introductions were used in the base population from which they were developed. Even so, it is probable that selected plants from falcata-sativa hybrids were major components of both.

Exotic Germplasm

What do we mean by the term, exotic germplasm? To me it represents sorts from the falcata-sativa-glutinosa taxonomic complex that have not been used or sorts that are not now used or sorts that are just coming into use in breeding and includes:

1. Commercial sorts and cultivars new to the geographical area of interest, i.e. DuPuits et al in the early 1950's to the Northern U.S. and Canada. These are tetraploids and may be used directly as cultivars or as parents in developing populations for selection.

2. Wild and naturalized populations from different countries and/or ecological zones. These too are tetraploid. M. sativa populations of this sort exist from the Mediterranean, the Near East, Central Asia, Northwest India, Mongolia, China, Manchuria and Korea to the Pacific. Naturalized populations from South America, Australia and South Africa exist. The use of Turkestan alfalfa for resistance to bacterial wilt in the 1930's is an example.

3. Wild tetraploid M. falcata which co-exists with the northern edge of the distribution of M. sativa and in a belt to the North of this. These have been used in hybridizing with M. sativa in nature and by breeders.

4. Sand lucerne, tetraploid hybrid populations, that exist where the two parental species, sativa and falcata coexist or meet. Such populations are represented in land races and cultivars from Central Europe and European Russia.

5. Unadapted tetraploid sorts used in hybridization with adapted sorts. An example is the use of selections from a cross of M. falcata x Hairy Peruvian in the breeding of Atlantic.

6. Diploid species including M. sativa, M. falcata, M. coerulea and others from the falcata-sativa-glutinosa complex. In the USSR several species are described in the diploid M. falcata group. These diploid yellow-flowered sorts have been used in breeding but not the diploid blue-flowered ones. The diploid falcata x tetraploid sativa hybrids have been important in the breeding of Rhizoma, Narragansett and Vernal and many cultivars that have originated from them. These hybrids perhaps have been important in the breeding of the creeping rooted varieties from Swift Current, Saskatchewan and the varieties from South Dakota, Teton and Travois. Perhaps other variegated sorts have arisen by this method; a tetraploid hybrid from an unreduced gamete from the diploid and a

reduced gamete from the tetraploid.

7. Other species including diploids, tetraploids and hexaploids. At various times Russian taxonomists have described over 100 species of perennial Medicago. Lesins in the 1972 Alfalfa Monograph describes 62 species (54 good ones).

Use of Exotic Germplasm In Our Laboratory

1. Commercial sorts and cultivars new to the area:

DuPuits and Narragansett and similar sorts. Narragansett was identified in a Uniform U.S.D.A. Nursery Trial planted in 1946 under the designation A-197. DuPuits was identified in a Uniform U.S.D.A. Plot Trial planted in 1948.

To understand to some degree the origin of Narragansett I shall review the origin of Rhizoma which originated from 6 F1 hybrid plants produced at the University of British Colombia from the seed produced on one clone of M. falcata obtained from South Dakota by Professor Klinck in 1914. In 1921 250 seeds were obtained on the >Don= clone and 6 plants from these were hybrid as determined by flower color and pod shape. The available pollen sources were plants of Grimm and Ontario Variegated. The 6 hybrid plants and their progeny were the subject of Professor Moe=s doctoral thesis in the Department of Plant Breeding at Cornell to 1928. He found their progeny were the subject of Professor Moe=s doctoral thesis in the Department of Plant Breeding at Cornell in 1928. He found their progeny to segregate widely for many characteristics including fertility. The mean of 30 plants from each clone produced the following results:

Chromosome
Clone Seeds/Flower Number 2n =

H-7 2.00 32
H-56 1.70 24
H-68 1.15 24
H-71 .45 32
H-156 1.48 32
H-190 1.30 32

G. G. Moe practiced mass selection in the progeny from these plants and released Rhizoma in 1948. The chromosome numbers were not determined until R. A. Nilan studied them for a Master=s thesis at the University of British Columbia and published the results in 1951. The Don falcata was identified as a diploid, 2x = 16.

Ledingham, working with R. A. Brink, had determined in 1940 that tetraploid and triploid hybrids could be produced by hybridization between diploid falcata and tetraploid sativa and selections from these were important in the breeding of Vernal which was released in 1953.

The plants from Moe=s thesis were continued in the nurseries at Ithaca by Professor C. H. Myers of the Department of Plant Breeding. In 1932 Professor Odland of the University of Rhode Island visited these nurseries and was very impressed with the hybrids from British Columbia. He obtained several clones and these became the basic materials for Narragansett. The variety was first tested in USDA trials as A-197 and released in 1952 because of its outstanding performance in New York, Pennsylvania and New England.

DuPuits was soon identified from the 1948 and subsequent trials to be outstanding for short-time stands on well drained soils in New York and adjacent areas and was recommended for use about 1955. At that time it was felt that French alfalfa was poorly adapted and strong recommendations were made against its use.

The alfalfa breeding program at Cornell was reactivated in 1945 when S. S. Atwood joined the faculty from Northeast Regional Pasture Research Laboratory. Much effort went into the development of synthetic varieties from the U.S.D.A. program centered at Lincoln, Nebraska and several populations from the University of Wisconsin. None of these proved worthy of release and the first pedigreed alfalfa varieties for the Northeast with clear superiority over Grimm, Ranger and Commons were Narragansett, DuPuits and Atlantic in 1949.

From Narragansett we have produced Mark II (improved seed production) and Iroquois (bacterial wilt resistance). The latter variety was developed by the backcross method with Vernal serving as the non-recurrent parent.

From DuPuits primarily but also two other similar sorts, Socheville and Flamande we have produced Saranac (bacterial wilt resistance), Honeoye (higher yield) and Saranac AR (anthracnose resistance). Saranac was developed by the backcross method with A225, the Northeast Synthetic, as the non-recurrent parent. Honeoye was developed from three cycles of recurrent selection from Saranac for vigor and variegated flower color. Saranac AR was developed by one cycle of recurrent selection from the U.S.D.A. germplasm release, GP18 Belts. 2-An4, for winter survival at Ithaca, bacterial wilt resistance and anthracnose resistance. These new sources of germplasm, Narragansett and DuPuits, have truly contributed much to alfalfa improvement in the Northeast and adjacent areas.

2. Wild and naturalized populations of M. sativa from the centers of origin.

These have contributed specific genes and characters to U.S. cultivars. Bacterial wilt resistance and winterhardiness from the Turkistan alfalfa were important in the development of Ranger and some subsequent cultivars. We have included these materials directly and through the U.S.D.A., Lincoln, Nebraska nurseries from the beginning. Six of the 10 clones in the cultivar, Cayuga came from the U.S.D.A. Nebraska germplasm, the other 4 clones came from old stands in New

York. This cultivar, released in 1962, was clearly superior to Ranger in the Northeast. It was subsequently replaced by Saranac and Iroquois.

On several occasions we have grown a large collection of Plant Introductions. In the beginning we isolated nothing of value. Several were described as showing significant degrees of resistance to foliar diseases and insect pests, vigor, winterhardiness, etc. These were rarely verified in our observations. With the advent of the alfalfa weevil, I made another effort in 1965 to observe a large collection of introductions, primarily tetraploid M. sativa. A few tetraploid falcata hybrids and a few diploids were also present. Few noteworthy selections have originated from this material. However, some introductions from Afghanistan showed some resistance to bacterial wilt and these along with several from Turkey, Iran and India which showed good vigor and winter survival were included in a recurrent selection program. A population has been developed from these which approaches Saranac in performance. It was developed by three cycles of recurrent selection. It will be released as germplasm to interested breeders and not as a cultivar.

How have we coped with these introductions? In the 1965 nursery there were over 400 plant introductions along with about 300 other seed lines from our breeding materials grown in 5 replications of 10 plants per replication. The 700 lines were bulked into the following populations:

25 P.I. groups
9 U.S. and N.Y. groups
6 Canada groups
5 germplasm groups
7 N.Y. cultivar checks
52

Open-pollinated seed was harvested from the surviving plants bulked from within each group. These have been grown once more and open-pollinated seed has been harvested again. Thus we have exotic lots with introgression from domestic lots and other exotic lots and vice versa.

3. Wild tetraploid M. falcata.

We have not used this material to any extent, all lots have proved to be mixed with blue-flowered sorts and very susceptible to bacterial wilt. Some of the variegated flowered sorts in the previous group have been used to a greater extent. However, a release from Swift Current by D. H. Heinrichs, Sc Syn ML 3713, is relatively pure and is being introduced into our program.

4. Sand lucerne or tetraploid hybrid populations.

These have about the same status in our program as the tetraploid M. falcata. Most of those from Germany and Central Europe have been very susceptible to

bacterial wilt. However, they are represented in the group from the Plant Introductions referred to before.

5. Unadapted tetraploid sorts.

We are exploring the possible value of a few of these in crosses to our cultivars. We expect to backcross to our cultivars and select for earliness, vigor, etc.

6. Diploid species including the blue-flowered M. sativa and M. coerulea and one M. falcata, P.I. 258754, from the above mentioned introduction nursery which has some resistance to bacterial wilt and has been very persistent in our nurseries. We are looking at hybrids at the 2x, 3x, 4x and 6x ploidy levels. Since these diploids are presumably the parents of tetraploid alfalfa, they may have important germplasm to add to that in our current cultivars. It seems to us important that the germplasm base of cultivated alfalfa be broadened and the use of wild species and, possibly the higher level of ploidy (6x) are promising ways to do this. We have developed limited populations of hybrids at the above mentioned ploidy levels. Although good cultivars of alfalfa currently exist, there is some consensus that breeders are approaching a yield ceiling with current germplasm. There is also some evidence that the range for forage quality constituents (for which breeding is just beginning) may be relatively narrow in existing germplasms. These seem to us to be compelling reasons for broadening the germplasm base in the hope of further improving this valuable species.

Some of the methods we are investigating include:

1. Use of 2x M. sativa, USDA and Sask S-2128, as a parent of 4x hybrids. We have populations from the intercross and backcross to 4x from over 50 F1 tetraploid hybrids. These hybrids are often rather prostrate and intermediate in vigor.

2. Use of 2x M. coerulea, Ottawa 1893 6508 USSR, in a similar way. This M. coerulea has an interesting characteristic of extensive branching. The F1 hybrids are very vigorous.

3. Use of 2x M. falcata, P.I. 258754, as a parent of 4x hybrids. We have populations from the intercross and backcross to the 4x from over 35 F1 tetraploid hybrids. These populations look very promising.

4. Use of 6x hybrids to be developed from triploid hybrids of the above crosses of 2x x 4x in developing a hexaploid source. We have several triploid hybrids and have produced two hexaploid hybrids by the use of colchicine. We have naturally occurring hexaploids from Saranac obtained from E. T. Bingham, University of Wisconsin. We have also shown that hexaploids can be produced from 3x x 6x crosses by the unreduced gamete technique. As one can see there are many combinations that can be developed. It may be possible to produce hexaploids with 16 chromosomes from each of 3 diploid species: sativa, coerulea and

falcata. In addition it may be possible to introduce the natural hexaploid species, M. cancellata into these populations. It is a perennial and a few F1 hybrids with tetraploid alfalfa have been reported.

Murphy sent this paper to H. M. Tysdal, T. E. Odland, and H. B. Sprague to verify the information on Ranger, Narragansett, and Atlantic, respectively. The following are excerpts from the letters he received.

Tysdal wrote: I have read your article with great interest, and believe you have done a good job to bring these things together, and to point out some of the possibilities. While I have long been removed from the scene of action, it is interesting to note developments. You certainly have put out several fine alfalfa varieties.

You were kind enough to ask for Acorrections or additions...@..I am not in position to suggest very much, but a few thoughts have occurred to me which I might pass on:

With respect to Aexotic@ species, has anyone ever tried crossing alfalfa with M. lupulina or similar species? Some of the lupulinas must carry a high degree of resistance to leaf diseases. With the various numbers of chromosomes perhaps such a wide cross would be possible.

Another thing I noted, and perhaps it was outside the scope of your paper, is no mention of hybrid alfalfa. Is this out the window? Perhaps with alfalfa one should not think of strictly an F1 hybrid; but it seems to me that several highly selected, self-sterile, highly compatible plants, increased vegetatively and planted for seed production could produce a very superior variety. Included in these would be one or more upright, spreading types. Such a variety could not be called a hybrid, but a APolycross variety@ seems to be the best name I can come up with. Vegetative propagation should present no problem. The control of volunteers in the seed production field should be controlled.

If the volunteers could be completely controlled, perhaps a male sterile could be used and an F1 cross produced.

Your reference to the Nebraska work is very nice. I believe Ranger represented the first alfalfa variety which definitely brought in a specific gene or genes for disease resistance from a foreign variety, still maintaining the desirable characteristics of the domestic varieties. One thing that was not often brought out is that Ranger was a very good seed producer. Turkestan was notoriously poor, and I have often heard the California seed growers mention that Ranger was a real good seed producer, even compared to domestic varieties.

While I am on the subject, I think one of the major contributions was to prove that alfalfa flowers required tripping to produce seed, which was not at all accepted at first. This resulted in an increase in seed production from an

average of 60 pounds per acre to over 1,000 pounds per acre in seed producing areas. That is even better than hybrid corn. Along the lines discussed in your paper, I believe I was first to draw attention to the fact that alfalfa is a tetraploid.

Odland wrote: The material in the manuscript is presented in a precise, well organized and clear manner. Everything is Aright on the button.@ I have no revisions or corrections to offer. I appreciate your kind remarks about Narragansett and my part in its development. I was especially pleased that you cite Dr. Moe and his outstanding achievements in the early alfalfa breeding work. Both Narragansett and Vernal owe much of their lineage to falcata hybrids.

I am also pleased that you mention my good friend and counselor, Dr. Myers. I shall be forever grateful to him for so kindly and generously sending me some of his choice clones that I admired when I saw them in his alfalfa nursery. I used them extensively in laying the foundation for Narragansett.

Finally, I want to congratulate you, Murph, on the fine work you and your staff are doing in the field of plant breeding. You are adding luster to an already bright and shiny escutcheon.

H. B. Sprague wrote: Your statements about AAtlantic@ alfalfa are essentially correct. I collected a fairly large number of alfalfa strains in the mid-1930s including many from the USSR plant breeder at Tashkent, Turkestan. I added all the available North American strains and varieties. Also, I included some falcatas from North India that my sister had collected at my request. The subsequent falcata hybrids were easily identified. Some of the more promising were added as new strains. Every strain or variety was carried as a family but there was no control of pollination in the nursery. The Hairy Peruvian crosses were a side issue, but they were added to the general breeding nursery. Each strain was maintained by selection in each generation of a few outstanding plants on the basis of vegetative vigor (plant wt.) and abundance of seed production. I grew some 5 or 6 plant generations before I left for military service in 1942. Atlantic was the principal output of this program, being the composite of all rigorous strains in the entire breeding nursery. So far as I know, there was no further selection in the composite.