Proceedings of the

36th
North American Alfalfa

Improvement Conference

Bozeman, Montana

August 2-6

1998

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What are Annual Medics?

Gary R. Bauchan
USDA-ARS, Soybean & Alfalfa Research Lab., Beltsville, MD 20705-2350

Medics are annual species of *Medicago* which are closely related to alfalfa. The annual medics are endemic to the Mediterranean region of the world. The medics are fast growing and true annuals were most of them will complete their life cycle in 65 to 100 days. Medics appear to be best adapted to soils with a pH of 6 and above, however, some species are more tolerant to low pH soils such as *M. murex*. Medics have been found to produce up to 200 pounds of nitrogen per acre provided effective inoculant is used. Medics should be inoculated with a appropriate *Rhizobium* as the standard strains used for alfalfa may not be effective. Medics can be cut for hay with an average yield of 1-3 ton/acre with a single cut taken at 60-70 days after planting. Because medics tend to lodge and may be difficult to cut and bale, grazing may be a suitable alternative. If the medics are grazed high with some stems remaining some regrowth may occur. Medics have quality potential similar to alfalfa which is influenced by the stage of maturity. They will cause bloat and need to be introduced slowly as part of a total ration or mixed with grasses. The annual medics are not cold hardy and they will die after a killing frost. Snail (*M. scutellata*) and gama medics (*M. rugosa*) have a natural resistance to the alfalfa weevil and the potato leafhopper due to glandular tipped hairs on the stems, leaves and pods. All of the medics have yellow flowers and are self pollinating, therefore they do not require bees for pollination. The seed pods are very unique and distinct with seeds larger than alfalfa. Typically the medics have greater seedling vigor than alfalfa especially the large seeded species. The seeds can remain viable in the soil for long periods of time and are thus able to reseed themselves after a period of time. However, if the medics are grown in areas where there is adequate moisture, softer seed will be produced and a second generation may be produced in a single growing season. The annual medics are used extensively in Australia on over 30 million hectares. They are utilized as a winter annual to improve soil structure, increase soil nitrogen and as a source of forage. The species most widely grown in Australia are: *M. littoralis*, *M. polymorpha*, *M. trucatula*, *M. scutellata*, *M. rugosa*, and *M. italica* (Crawford, 1989). Renewed interest in sustainable agriculture systems has placed renewed interest on legumes. Annual medics may have the potential of being utilized in various sustainable systems. Some potential uses are: 1) pastures, either in a lay farming application or permanent pastures, 2) green manure produced as green fallow or cover crops, and 3) companion or smother crops for weed control. The Australian medic collection contains over 21,000 accessions and the U. S. collection contains 3,000 accessions A core collection has been designated for both collections which is composed of 1705 and 211 accessions, respectively (Skinner et al. 1998 and Diwan et al., 1994) These core collections can be utilized as a small subset of the larger collections which can be evaluated for local adaptation and potential utilization.

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Recent Germplasm and Collection Activities in the Australian Medicago Genetic Resources Centre

Geoff C. Auricht and Steve J. Hughes

South Australian Research & Development Institute, Adelaide, SA, 5001

The Australian Medicago Genetic Resource Centre (AMGRC) is maintained by the South Australian Research and Development Institute in Adelaide, Australia. The Centre has a forty year history and houses 34,500 accessions of over 100 genera and 600 species from 52 countries. This includes the Worldís largest Medicago collection with some 23,000 annual medics and 1,500 perennial accessions. The other accessions comprise 8,000 other pasture legumes covering 45 genera along with 2,000 pasture grasses.

Recent Activities:

1)Collection

The AMGRC collection is growing by an average of 830 accessions each year. These are obtained mostly from collection activities involving Australians. Recent collections include:

Date	Location	Visiting Collectors	Genera	AMGRC Accessions	Sites
Jun-98	Greece	Snowball & Maxted	11	430	53
Jul-97	NE Turkey	Hughes & Reed	10	254	31
Jun-97	South Turkey	Maxted	7	145	44
Jul-96	East Turkey	Auricht & Lamont	12	157	42
Jun-96	SE Turkey	Bennett & Maxted	9	204	29
Jun-95	Greece	Nutt & Carr	2	25	25
Jun-95	Spain	Enneking	2	32	27

These collections have been coordinated through the Centre for Legumes in Mediterranean Agriculture (CLIMA), University of WA, Nedlands, WA.

2) Characterisation

The AMGRC annually grows some 1,000 accessions for characterisation and preliminary evaluation. Accessions are grown in 4m rows containing 25 spaced plants. In recent years the emphasis has shifted from *Medicago* to other genera. Activities are summarised in the following table:

Year	*Medicago*		*Trifolium*		Other Genera*			Total
	Species	Accessions	Species	Acc.	Genera	Species	Acc.	
1998	10	30	41	160	12	93	428	618
1997	8	98	5	58	16	55	723	879
1996	8	948	0	0	2	3	229	1177
1995	15	1084	0	0	0	0	0	1084

*Includes *Astragalus*, *Hymenocarpos*, *Lotus*, *Scorpiurus* and *Trigonella.*

Data on over 30 characteristics is recorded from these rows. Seed is harvested and samples stored to international standards at -20°C and 2°C.

The AMGRC has an extensive database of passport, characterisation, seed inventory and other collection details. More information is available on the SARDI web site (www.sardi.sa.gov.au/livestck/pastures/pastures.htm).

Future genetic resources plans include a *Rhizobium* collection in North Africa and a pasture legume collection missions in Central Asia and China.

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Development of a core collection for the Australian annual medic collection

D.Z. Skinner,[1] G.R. Bauchan [2], G. Auricht [3] and S. Hughes [3]
[1]USDA-ARS, Manhattan, KS and [2]Beltsville, MD; and [3]South Australian Research & Development Institute, Adelaide, South Australia.

A collection of 20,997 annual Medicago accessions, representing 34 species and 42 subspecies, was evaluated for 27 agronomic characteristics from 1968 to 1994. We have developed a method to assemble a core collection from these accessions by maximizing the selected diversity from within groups of accessions defined by species, subspecies, and geographic origin.
The mean Euclidian distance of each accession to all other accessions was calculated; passport data was used to group the accessions by species, subspecies, and geographical origin yielding 793 groups. Each group was represented in the core collection by the extremes of average Euclidian distance found within the group. A small average Euclidian distance indicated "typical" accessions, while a large average Euclidian distance indicated the more unusual types. Groups comprised of one or two accessions were included as a unit.

The mean Euclidian distances calculated ranged from 5.4 to 48.7. Retaining 0.5% of the typical end and 3.0% of the diverse end of each of the 793 groups resulted in the retention of 1705 accessions, or 10.4% of the entire collection. To evaluate how well this 1705 accession core collection captured the variation in the original collection, the ranges of each character in the core collection were compared to the corresponding ranges in the entire collection. Both extremes were captured in 20 of 27 characters. An additional accession to represent each of the extremes not included, easily could be added to the core collection.

Associations of trait expression were evaluated by calculating correlation coefficients of all combinations of the evaluated traits. Because the proportion of variance in one trait that can be attributed to its linear relationship to a second trait is indicated by the square of the correlation coefficient, we suggest that meaningful correlation coefficients in this case are those with absolute values greater than 0.71, i.e.more than 50% of the variance of one trait is predicted by the other. By this criterion, the relationships of interest are: seedling vigor and average winter yield (r=0.74), average spring yield and average winter yield (r=0.78), grams of seed per plant and grams of pods per plant (r=0.91), number of seed per plant and number of pods per plant (r=0.74), 1000 seed weight and 1000 pod weight (r=0.75), number of seeds per plant and grams of seeds per plant (r=0.77), florets per peduncle and pods per peduncle (r=0.86). None of these relationships were surprising, but they do suggest that it may not be necessary to measure all of the traits involved in future germplasm evaluations.
The core collection assembled here very likely represents the great majority of the genotypes present in the annual medic collection, and provides a resource for efficient further development of medics as part of an agricultural system.

Australia's Ley Farming System: Can It Be Adapted to the U.S. Great Plains?

Robin W. Groose

Plant Sciences Dept., Univ. of Wyoming, PO Box 3354, Laramie WY 82071 USA groose@uwyo.edu

On the semi-arid U.S. Great Plains, summer fallow is a dominant practice in the winter wheat agroecosystem. Fallow has stabilized wheat yields but jeopardizes long-term economic and ecological sustainability in many ways, including: low water use efficiency as only 20-40% of precipitation during the 14 month fallow period is stored for the subsequent wheat crop; soil erosion by both water and wind resulting in soil loss and pollution of water and air; loss of soil organic C and N; leaching of fertilizers and pesticides into ground water; generation of saline seeps; and only one harvest every two years. More intensive farming that includes annual legume pastures in the rotation may prove more profitable for producers at the same time that it solves problems of ecological sustainability.

In semi-arid southern Australia, legume pastures have replaced fallow and integrate dryland crop and livestock production. Literally, "ley" means "meadow," and "ley farming" is to grow crops in rotation with self-regenerating annual legume pastures. In the ley farming system, medic pasture (annual species of the genus *Medicago*) alternates with wheat in a two year cycle. Medics regenerate yearly from a soil seed bank, and in the pasture phase of the cycle provide forage for livestock. In the cereal phase, regenerating medics may briefly furnish forage before seeding the cereal crop.

In America, a ley system might provide many benefits including (1) more profitable cereal production, (2) quality livestock forage, (3) self-regenerating pastures, (4) integrated pest management, (5) reduced fertilizer inputs, (6) improved water use efficiency, (7) improved water quality, (8) improved air quality, (9) soil conservation, (10) improved soil quality, (11) no need for strip farming, and (12) global benefit of increased carbon fixation.

The University of Wyoming, in collaboration with the South Australian Research and Development Institute, is conducting research on ways in which annual *Medicago* spp. might partially replace fallow either as "summer medics," regenerating and completing a life cycle within a single summer growing season before wheat planting or after wheat harvest, or as "winter medics," regenerating under or after wheat, overwintering, and completing the life cycle the following spring and summer before planting of the subsequent wheat crop.

In this paper I summarize plant breeding research towards achieving a **WINTER MEDIC IDEOTYPE** for the Great Plains comprised of six traits. **(1) Winterhardiness.** *Medicago rigidula* and *M. rigiduloides* have proven to be the most winterhardy annual medics in Wyoming. Winter desiccation rather than cold per se appears more lethal to medics. **(2) Nitrogen Fixation.** These species differ markedly in *Rhizobium* specificity, although distinct within-species differences are also evident. *M. rigidula* is effectively nodulated with commercial *M. sativa* inoculum. **(3) Regeneration**. High levels of hardseededness are a prerequisite to annual legume persistence through crop rotations and both *M. rigidula* and *M. rigiduloides* produce hard seed in Wyoming. Breakdown of hardseededness and effectiveness of regeneration from soil seed banks will require many years of evaluation. **(4) Competitiveness.** Medics must compete with aggressive weeds on the Great Plains. Although well-established medics can suppress weeds, successful establishment and regeneration of medics may prove to be more a function of wheat, residue, weed and grazing management than of genetics. **(5) Forage Production.** Insofar as medic pasture replaces fallow which provides little grazing except for wheat residue, this is an "easy" trait. (**6) Early and Prolific Seed-Set.** Better lines of *M. rigidula* and *M. rigiduloides* survive winter to produce sufficient seed to sustain a soil seed bank. Seed is mature by early July to provide a brief period of true fallow before wheat planting.

Management options for pasture ley-cereal rotations in south-eastern Australia

R.A.Latta and N.Hill
Agriculture Western Australia, Katanning, Western Australia 6317

Self-regenerating annual medics (Medicago spp.)in pasture-crop rotations may benefit both cereal and livestock production through fixing nitrogen, providing a disease break for cereals, and improving livestock fodder supply (Latta and Carter, 1998). These benefits are often reduced due to annual grasses in the pasture. This study examined grass control options in a 3 year pasture-pasture-wheat rotation with a mechanical fallow and a selective grass herbicide. In a replicated experiment 375 ml/ha fluazifop (500 ml/L a.i.) applied in August 1994 and a mechanical fallow treatment, commenced in July 1995 was compared on pasture and cereal production in a low rainfall region of south-eastern Australia. Sheep grazed plots at 1.25 DSE/ha in 1994 and 4 DSE/ha in 1995. Plots were sown to wheat in June, 1996, and grain yields and protein percentages measured at harvest in November. Rain in the 3 years totalled 164, 403 and 333mm. The average is 338mm. The densities of established medic plants in 1994 and 1995 were similar (200 to 300 m-2). There was a lower grass density in 1995 due to 1994 selective grass control. Pasture production was similar until the mechanical fallowing in July 1995. This also resulted in lower densities of medics regenerating in 1997 following the 1996 wheat crop (Table 1). The chemical grass control treatment had higher volunteer grass densities in the wheat phase of the rotation. This was reflected in a lower grain yield (Table 2). The use of costly selective grass herbicide almost 2 years prior to a cereal crop is desirable to ensure cereal root disease control, maximise legume production and extend the period of livestock fodder (Latta and Carter, 1998). Fallowing resulted in a higher grain yield, however, it also necessitates re-establishing a medic pasture following the cereal phase. Community concerns relating to soil erosion will ultimately force the abandonment of this practise irrespective of economic considerations.

Table 1. Cumulative pasture production (kg DM/ha) in the winter and spring of 1994 and 1995, and the medic plant regeneration (plants/m^{-2}) in 1997.

	1994		1995		1997
Treatment (year applied)	Winter	Spring	Winter	Spring	Regeneration
Mechanical fallow (1995)	270	703	2090	0	27
Sel.Grass Control(1994)	230	898	1980	4250	102
L.s.d. (*P*<0.05)	n.s.d.	n.s.d.	n.s.d.	N/A	16.1

Table 2. Annual grass density (plants/m^{-2}) wheat grain yields (t/ha) and protein (%) in 1996

Treatment	Annual grass	Grain yield	Protein
Mechanical fallow	5	2.6	12.8
Sel. Grass Control	50	2.3	11.6
L.s.d. (*P*=0.05)	25.5	0.236	n.s.d.

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Increased nutrition and disease control improve medic pasture production

William Bellotti[1], James Neal[1], Alan McKay[2] and Mingpei You[3]
[1] The University of Adelaide, Roseworthy, South Australia, 5371 [2] South Australian Research & Development Institute, Adelaide, SA, 5001 [3] CLIMA, University of Western Australia, Perth, Western Australia, 6009

In recent years pastures based on annual medics have not met the expectations of many low rainfall farmers who rotate wheat or barley with pasture. Farmers report dissatisfaction with the low productivity of the annual medics and the poor growth of wheat following medic dominant pasture. Several contributing factors have been implicated in this scenario including; fungal root diseases, root nematodes, low soil fertility, herbicide residues, and low profitability of livestock enterprises relative to cropping enterprises.

Recent surveys have identified a number of common annual medic pathogens. In South Australia (SA) and Victoria (Vic) the most common fungal pathogens were *Rhizoctonia solani*, *Pythium irregulare* and *Fusarium* spp. The most common fungi in Western Australia (WA) were *Pythium* spp., *Fusarium* spp. and *Phoma medicaginis*. The root lesion nematode, *Pratylenchus neglectus*, was common in SA and Vic but in WA only 5 out of 116 sites had high nematode numbers. Soils supporting annual medic pastures in the SA and Vic study region are normally alkaline, calcareous, and often deficient in P and Zn. In the WA study region, soils supporting medics are often slightly acidic.

On-farm field experiments were conducted in 1996 and 1997 over seven locations. The design included four treatments:

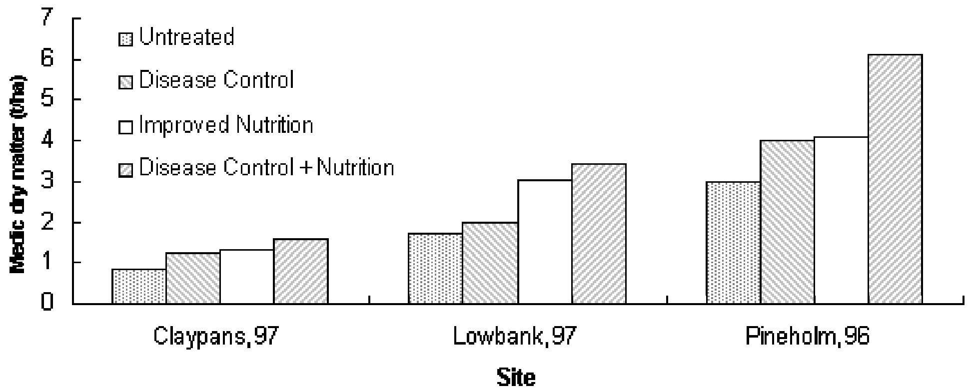
1. control: untreated medic pasture regenerating from soil seed bank

2. disease control: nematicide and mixture of three fungicides

3. improved nutrition: 31 kg P/ha and 8.16 kg Zn/ha

4. combination of treatments 2 and 3

Despite different site and seasonal conditions, the response of medic dry matter at flowering to the treatments followed a typical trend (Figure), the combined improved nutrition plus disease control treatment often producing double the dry matter of the untreated control treatment.



These results demonstrate that large potential gains in productivity of annual medic pastures are possible. Practical solutions to realise this potential require ongoing research and development.

Breeding and Selection of Annual Medics for Resistance to Pests, Particularly Alfalfa Aphids.

Andrew W.H. Lake,
Pasture Legume Breeder, 1 Kingston Avenue, Daw Park, SA, 5041, AUSTRALIA

Annual medics, principally cultivars of the barrel (*Medicago truncatula*), strand (*M.littoralis*), burr (*M.polymorpha*) and disc (*M.tornata*) medic species, have been widely used in Australian ley farming systems since the 1950's. However, the arrival of both spotted alfalfa aphid (SAA) and blue-green aphid (BGA) in Australia in the late 1970's resulted in massive damage to medic pastures throughout the continent, with all cultivars available at that time being highly susceptible to one or both pests. In consequence, considerable effort has been devoted to the breeding and selection of multi-aphid resistant cultivars.

Phase 1; cultivars developed directly from introductions.

Mass screening for SAA and BGA resistance of about 12 000 accessions in the medic species mentioned above resulted in the identification of a small number of barrel medic lines only with resistance to BGA and some resistance to SAA. Out of the 12 000, less than 0.1% exhibited sufficient resistance to warrant field test, but from these, the cultivars Paraggio (1984), Sephi (1986), and Parabinga (1987) were released.

Phase 2; cultivars developed by hybridisation and selection.

Unlike alfalfa, medics are very highly self-fertile, generally diploid and technically difficult to hybridise, so that breeding and selection systems for these annuals are quite contrasting to the perennials. On the minus side, this necessitates medics needing to undergo generally *at least* 6 generations of segregation post hybridisation before lines with acceptably high levels of homozygosity can be isolated. On the plus side, resistance can be fixed in all plants of a cultivar, and cultivars are much more readily definable and distinguishable from each other.

Paraggio, Sephi and Parabinga all have significant agronomic deficiencies, and hence efforts were subsequently focussed on breeding and selection of resistant cultivars without those weaknesses. This process has been highly successful; SAA and BGA resistance genes have not only been moved around within the barrel medic species, but have also been transferred into strand and disc medics. The success of this process can be gauged against the fact that the newer, bred cultivars produce up to 20% more herbage and as much as 40% more seed than the cultivars they replace in the first year alone; even in the absence of aphids. The cultivars in this category are Harbinger AR strand (1990), Mogul barrel (1992), Caliph barrel (1994) and Herald strand (1996) medics. Pending (unnamed) aphid resistant releases include another barrel, as well as a disc medic.

With respect to the North American scene, it appears likely from the Australian experience that pest resistance, particularly to the already present alfalfa aphids, will be an advantage for at least some regions.

Annual medics and rhizobia research in Australia

William Bellotti[1], Ross Ballard[2], Jo Slattery[3] and John Howieson[4]
[1]The University of Adelaide, Roseworthy, South Australia, 5371, [2]South Australian Research & Development Institute, Adelaide, SA, 5001, [3]Agriculture Victoria, Rutherglen, Victoria, 3685, [4]Murdoch University, Perth, Western Australia, 6014

Annual medics (primarily *Medicago truncatula*, *M. littoralis*, *M. polymorpha*, and several other less important species) form the basis of the majority of pastures grown on neutral to alkaline soils in the low to medium rainfall zone (250 - 500 mm annual rainfall) of the Australian wheatbelt. The general practice on these soils is to sow uninoculated seed and rely on naturalised *R. meliloti* strains for nitrogen fixation. In Western Australia (WA), annual medics (primarily *M. polymorpha*) are also grown on slightly acid soils. An overview of current research on the *Rhizobium meliloti* - annual medic symbiosis is outlined.

<u>Surveys of Rhizobia meliloti population size and effectiveness</u>

Recent surveys (Slattery et al. 1998) have confirmed the importance of soil pH on the population size of *R. meliloti*. Populations are generally high (>1000 per g soil) on neutral to alkaline soils (pH Ca > 6.5). This relationship leads to the situation of generally large naturalised populations in South Australia (SA) and Victoria (Vic), whereas WA soils generally support low populations. The effectiveness (ability to fix nitrogen) of naturalised populations is strongly dependent on the host legume, but for most of the dominant commercial species, effectiveness is usually adequate, although wide variation exists.

<u>Specificity of the annual medic - rhizobia symbiosis</u>

Annual medics vary in their ability to form effective symbioses with different *R. meliloti* strains. Some species (eg. *M. littoralis*), are highly specific in their strain requirement, whereas other species (eg. *M. truncatula*) are able to form effective symbioses with a wide range of strains. The Australian inoculant industry accommodates species requirements by providing two inoculant subgroups. Strain WSM688 (marketed as 'AM') services all annual medics other than *M. littoralis* and *M. tornata*. Strain WSM826 (marketed as 'AL') services the latter two medics as well as lucerne. Improved strains are being sought to replace both incumbent strains.

<u>Persistence of introduced rhizobia inoculants</u>

In SA and Vic, one of the intractable problems has been the introduction of inoculant strains into often large naturalised populations that vary in their effectiveness with the sown host. This problem may be averted by;

1. selecting host genotypes on the basis of their ability to nodulate effectively with the naturalised rhizobia population,

2. selecting host species/cultivars, which are adapted to the edaphic environment, but do not form a symbiosis with the naturalised rhizobia.

In contrast, naturalised populations in WA are often low, and the development of acid tolerance in the medic - rhizobia symbiosis (Howieson and Ewing, 1989) has resulted in the expansion of annual medics in WA. New methods for strain identification (Richardson et al. 1995), and other molecular genetics methods, are being employed at a number of locations and promise to greatly improve our understanding of rhizobial ecology.

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Constraints to the introduction of Medics in French Mediterranean farming systems

Jean-Marie Prosperi and Joëlle Ronfort
INRA SGAP Montpellier - Domaine de Melgueil. 34130 Mauguio, France

Sheep rearing is the dominant livestock industry and an old component of farming systems in French Mediterranean regions. Although it suffers from low productivity and rural decline, it represents the only way to maintain human activities in such marginal lands [(1)]. The French Mediterranean zone, a large strip from 50 to 120km wide along the Mediterranean coast and the island Corsica, covers 12% of France (7 millions hectares). It receives mostly winter rains with an average annual rainfall of 700mm and is subject to dry summers. Mean temperatures are still colder than in other Mediterranean climates. While the perennial forage are well known and used in Mediterranean France, the potential of annual self-reseeding forage legumes has still to be explored for both intensive or extensive sheep rearing systems. A large number of species occur in native vegetation, but few of them are used [(2)].

For medics, Ley-farming could be the prominent farming system for European dry areas such as central Spain, but its interest is limited to France where annual rainfalls allow more sophisticated rotations with cereals. The use of medics as an annual spring forage legume - to produce hay in place of vetches or peas, or to graze them in mixtures with grasses or cereals - is yet another possibility, but only for intensive sheep production.

The best way to use medics in our country is by over-sowing them in rangelands or in degraded natural pastures to increase both forage production and improve quality. Animals fed on nitrogen-rich forage, such as medics, increase their consumption of ligneous vegetation. This increase in the consumption of ligneous allows, for example, a better control of bushes in fire-break areas. Several experiments have been conducted with sub-clovers and medics in forest areas in the South of France.

As non-agricultural uses, Medics and sub-clovers can also be inter-seeded in vineyards to control weeds development and to reduce soil erosion on slopping lands, or to limit nitrate and herbicide pollution in intensive fruit productions. Medics are also increasingly incorporated in mixtures sown to turn green the railways or highways slopes in Mediterranean part of France.

Besides the economic and human constraints attached with the low level of productivity of sheep rearing systems in the South of France, one of the main constraint is the availability of adapted materials. Our breeding activity aims at producing medic cultivars suited to these uses and adapted to our environment. To broaden the basis of the variability available, a large program of collections from western Mediterranean basin has been realized [(3)(4)]. Some results of the agronomic evaluation of our core-collection of *Medicago truncatula* and comparison with performance of Australian cultivars will be discussed.

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Overview and Agronomic Evaluation of the USDA Annual Medic Germplasm Collection

Richard C. Johnson and Walter Graves
USDA-ARS, Western Regional Plant Introduction Station, Pullman, WA 99164-6402

The USDA-ARS maintains a collection of medics consisting of 3,562 accessions representing 48 taxa. From 1987 to the present, regeneration and evaluation has been completed on this collection at Riverside, CA. Currently over 90% of 3,562 medic accessions are available for distribution. Evaluation data is available on roughly two-thirds of the accessions in the collection and is available from the Germplasm Resources Information Network (GRIN) (www.ars-grin.gov/npgs/).

For regeneration, accessions are germinated in small pots in a greenhouse during October and transplanted to irrigated field plots in winter. Normally, 50 plants per accession are established in a 6 m row. In the following spring/summer, dropped pods are vacuumed, threshed, cleaned, and shipped to Pullman,WA for storage and distribution. During regeneration, plants were evaluated for:

Winter vigor : visual scale (1=most, 9= least), determined on 2,466 accessions.
Growth habit : visual scale (1=most, 9= least), determined on 2,222 accessions.
Days to flowering: days from planting to first open flower, determined on 2,487 accessions
Above ground biomass : visual scale (1=most, 9= least), determined on 2,487 accessions
Seed production potential : visual scale (1=most, 9= least), determined on 2,481 accessions.

Weight per 100 seeds (g) has also been determined on 1,776 accessions. For the thirty-eight species examined during the regeneration process, a wide range of agronomic types were found within most species. Eleven of the most important species (*M. italica*, *littoralis, lupulina*, *minima*, *murex, obicularis, polymorpha, rigidula, rugosa, scutellata*, *truncatula*) were compared in more detail. *M. polymorpha* had the highest winter vigor and the fewest average days to flowering at 112. *M. lupulina* had the lowest winter vigor and the most days to flowering at 145. For growth habit, *M. scutellata* was the most erect and *M. littoralis* the most prostrate of the eleven species. Biomass production was highest for *M. murex* and lowest for *M. rugosa*. *M. rugosa* also had the lowest potential seed production with *M. obicularis* the highest. As expected, *M. scutellata* had the highest mean 100 seed weight (1.79 g) and M. minima had the lowest (0.12 g).

Canonical discriminate analysis was used to determine if distinct groupings would form using the evaluation data of the eleven species. (Seed weight data was excluded because of the significantly fewer observations available then for the other evaluation factors.) Some separation among species was observed, especially between *M. lupulina*, *M. polymorpha*, and a grouping made up of *M. scutellata* and *M. rugosa*. For the remaining seven species, the means of canonical functions were quite closely grouped and their standard deviations overlapped. The results suggested that the variability observed between many of the species was too small to allow distinct groupings. But since variation for the evaluation factors within species was high, selection within species appears possible.

Annual Medics in the Midwest

Craig C. Sheaffer
University of Minnesota, St. Paul, MN 55108

Annual medics (*Medicago* spp.) have been evaluated for a variety of uses in Midwest cropping systems. They are a good source of quality forage and N. Zhu et al. (1996) reported that some Australian medics produced 5.5 Mg/ha in a single harvest of forage when spring seeded and used as a short-season crop for harvest in fall or summer. Most annual medics had equal or higher crude protein and lower fiber concentrations than alfalfa. Shrestha et al. (1998) also reported that spring-seeded medic yielded 5.0 Mg/ha when harvested twice. Moynihan et al. (1996) evaluated medics as intercrops with spring barley (*Hordeum vulgare* L.) to provide ground cover and N for subsequent crops. Medics reduced fall weed biomass by 65% and contributed from 66 to 140 kg/ha of N in the fall, but also sometimes reduced barley yields. As an alternative to intercropping with a spring seeded small grain, Fisk et al. (1998, unpublished) no-tilled annual medics into wheat stubble following harvest and reported that accumulated above-ground N production ranged from 44 to 194 kg/ha of which 19 to 62% was from N2 fixation. Zhu et al. (1998) reported that spring seeded medic monocultures fixed from 100 to 200 kg/ha of N when harvested in August.

Medics have also been evaluated as intercrops with corn to suppress weeds, provide ground cover and add N. De Haan et al. (1997) seeded medics concurrently with corn and reduced weed dry weight production by 55% compared to a weedy control, but also reduced corn grain yield by 18%. Squire et al. (1998), showed that the effectiveness of annual medics as intercrops was increased when medics were seeded 28 days after corn planting. They found that the N fertilizer replacement value of annual medics for a subsequent corn crop was 36 kg/ha. Use of annual medics as intercrops with row crops is limited by unpredictable competition, inconsistency in favorable weather, and uncertain financial return.

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Annual medics (*Medicago* spp.) in irrigated corn for fall pasture in Eastern Wyoming.

Craig Alford, James Krall, and Stephen Miller
University of Wyoming, Laramie, WY 82071.

Farmers in the Great Plains have shown an interest in inter-cropping corn *Zea mays* with annual legumes. Advantages of farming systems which contain an annual legume include reduced soil erosion, improved soil fertility and improved aftermath forage quality. The objective was to first identify the most appropriate pasture legume species and then derive the most appropriate management system (seeding rates or seeding dates) for effective inter cropping in irrigated corn. To accomplish this experiments were conducted in 1996 at three sites (Huntley, WY; Lingle, WY; and Torrington, WY). Two of the sites were under sprinkler irrigation, the other was under furrow irrigation. Plots were 3.05 by 6.10 m with four replications in a RCB, split plot design. Eight legume species were evaluated in corn under both a weed free and weedy situation. In addition, there was a weed and medic free check and a weedy, medic-free check. Corn yields were reduced by the presence of medics in some treatments, others were comparable to the check yields. *Medicago lupulina* reduced corn yields by 4%, whereas *Medicago truncatula* reduced corn yields by 17%. Corn yields were reduced approximately 62% by the presence of weeds regardless of medic species. The medics did not significantly suppress weed growth. In 1997 two management systems studies were conducted at Torrington, WY, under sprinkler irrigation. Plots were 3.05 by 6.10 m with four replications. Main plots were medic seeding dates (2 weeks before, at, and 2 weeks after corn planting) and subplots a factorial arrangement of medic species (*Medicago lupulina* and *Medicago sphacrocarpus*), medic seeding rate (86, 172 and 344 PLS/m^2) and herbicide input level (low - imazethpyr + pendimethalin and high - imazethpyr + pendimethalin + bentazon). Corn silage yields were not influenced by any treatment. There were, however, significant differences between medic species, medic seeding rate, and herbicide input level on grain yield. Corn grown with *Medicago lupulina* yielded 6% more than *Medicago sphacrocarpus*. Corn yields were 16% and 13% higher when the medics were seeded with or two weeks after corn than when seeded two weeks before corn. The high herbicide input level increased grain yield 9% compared to the low input level. Corn yields were maintained while providing 2 t/ha of dry matter medic forage to the corn residue pasture resource. *Medicago lupulina* has been successfully established (92 plants/m^2) with corn in large plots for pasture trials this fall.

Effect of time of planting on the Growth and Development of Annual Medics (*Medicago* spp.) in Eastern Wyoming

M.J. Walsh, J.M. Krall and R.W. Groose
Department of Plant Sciences, University of Wyoming, Laramie, 82071-3354

Annual medic pastures have been used successfully for many years in ley-farming systems in areas with Mediterranean type climates around the world (Crawford *et al*., 1989). The ability of these pastures to produce high levels of quality forage under rainfed conditions has substantially improved as well as stabilised farm productivity of these regions. Annual medics appear to be highly suited for production in ley-farming systems in the dryland farming regions of North America. However, there is a need to determine the effects of the North American summer growing season on their phenological development. Thirteen annual medic varieties/species were evaluated for dry matter, pod and seed production as well as days from emergence to flowering and maturity at 3 times of planting in the 1997, growing season in Eastern Wyoming. Days to flowering and harvest are taken from the respective times of emergence which were for T1 20/5/97, T2 9/6/97 and T3 28/7/97. The dry matter production of the medic cultivars was related to the length of their growth and development period where increased periods resulted in higher levels of dry matter production (See Table). The Australian developed cultivars produced lower dry matter levels and had restricted pod and seed productions when compared with the winter hardy medic species (M. Rigidula, M. Rigiduloides and M. Lupulina). This was due to the generally shorter growth and development periods of the Australian cultivars, which were up to 2 months shorter than those of the winter hardy medics. The time to flowering from emergence for these Australian cultivars was also substantially shorter in the Eastern Wyoming than has been previously recorded under Australian conditions.

Table. Days to flowering and harvest and dry matter production of 13 annual medic varieties/species in Eastern Wyoming.

Variety	Species	Days to flowering				Days to harvest			Dry matter (t/ha)			Pod production (1000 pods/m^2)		
		T1	T2	T3	Australia*	T1	T2	T3	T1	T2	T3	T1	T2	T3
George	M. Lupulina	56	51	48		107	101	98	5.1	2.3	1.2	14.9	6.5	0
Sava[1]	M. Scutellata	41	36	27	71-80	58	51	48	1.3	1.7	1.8	1.5	2.0	0.9
10344	M. Rigidula	78	70	Frost		121	121	98	8.6	8.2	3.3	1.7	0	0
Santiago[1]	M. Polymorpha	43	38	41	71-183	71	6 0	76	1.1	0.9	1.8	1.3	8.5	0
Orion[1]	M. Sphaerocarpas	51	46	34		66	7 3	69	1.0	1.1	1.3	1.5	1.0	0
Caliph[1]	M. Truncatula	41	36	34	71-148	58	6 0	69	0.9	1.0	3.5	4.5	4.9	1.5
Herald[1]	M. Littoralis	43	38	41	101-110	10 0	8 7	76	3.3	4.9	2.1	12.3	11.3	0.5
Paraggio[1]	M. Truncatula	56	60	Frost	>110	91	87	76	3.9	5.6	3.0	6.0	3.2	0
119R-P	M. Rigiduloides	44	60	Frost		121	101	98	7.8	1.3	2.7	4.0	0	0
	LSD (p=0.05)								3.5	3.0	2.2	6.5	6.4	ns

* Days to Flowering in Australia (Crawford *et al*., 1989)
[1] Indicates Australian developed cultivars

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Snail Medic for Green Manure, Saline Seep, and Wind Erosion, and Grazing Black Medic with cattle in a Medic Pasture-Small Grain Rotation.

James R. Sims
Professor Emeritus, Montana State University, Bozeman, MT and Chief Agronomist, Timeless Seeds, Conrad, MT.

Selections were made periodically from 'Robinson' snail medic imported from South Australia in 1976. Selections were based on early growth during Montana spring weather and on forage (biomass) production. The variety 'Jamie' was released in February, 1998.

'Sava' snail medic was "late seeded" into summer fallow fields from 26 July to 8 August, 1993 at eight locations throughout Montanas' dryland grain producing areas where saline seeps were suspected to be likely to increase in size due to about 200%+ growing season rainfall. Stands were excellent, water was used and soils were protected from wind erosion throughout the fall/winter windy season.

Producers in central and eastern Montana report approximately 1 AUM/acre from Black Medic pastures maintained in rotation with annual crops, primarily wheat.

Burr Medic for Central and South Texas

W.R. Ocumpaugh
Texas A&M University Agricultural Research Station, Beeville, TX 78102

Forages provide 75 to 90% of the feed for the $7 billion Texas ruminant livestock industry. Traits required in a winter annual legume are: drought tolerance, good seedling vigor, hard seededness, and freeze tolerance. Burr medic (*Medicago polymorpha*) was introduced into Texas over 75 years ago and has become naturalized. It has all the above traits, almost to a fault, in that it is nearly a weed in my plot areas. In 1994, we collected seed of naturalized burr medic from eight locations in Central and South Texas with the objective of developing a superior, persistent, winter-growing variety. One collection was selected and two cycles of spaced-plant rouging has been completed. In 1996, we produced sufficient seed to evaluate our selection (BEPAS18) against three commercial annual medics from Australia ['Circle Valley' burr medic, 'Jemalong' and 'Parabinga' barrel medic (*M. truncatula*)] at 10 sites across Texas, Oklahoma, and Louisiana. BEPAS18 performed well in the high pH soils of Central and South Texas, and has never failed to re-establish itself in any planting in Central and South Texas. In fact, it spreads to colonize adjacent pastures, whereas none of the commercial medics seem to spread to adjacent pastures. Frost/freeze tolerance exceeded that of all commercial medics, but the northern limit of adaptability is somewhere south of Interstate 20. Total season dry matter yields of about 4000 lb/A measured at Beeville, College Station and Yoakum, TX are comparable to those of the commercial medics. Flowering of BEPAS18 is about a week later than Circle Valley burr medics and about 1 month later than Parabinga barrel medic, but about 1 week earlier than Jemalong barrel medic. Seed size (164,000/lb) of BEPAS18 is smaller than Circle Valley burr medic and Jemalong and Parabinga barrel medic. Seeds/burr is about four. In 1997 we established a seed production block under center pivot irrigation near Fowlerton, TX. This 15 acre block was established with three objectives--first to produce Foundation Seed, second to evaluate seed production under near commercial conditions, and third to demonstrate the utility of a Horwood Bagshaw vacuum seed harvester. At this time we have not recleaned and weighed all the bags, but this year's seed production is expected to be in the 900 lb/A range. We made a lot of mistakes in land preparation, so we were not able to harvest as much seed as we believe is possible. Hence, we believe that at least 1000 lb of clean seed per acre is obtainable under irrigation. Seed production and harvest costs will be in line with other annual forage legumes, as in a good day we could harvest over 3000 lb of seed. This legume will need to be planted only once (on sites where it is well adapted) at a seeding rate of 8 to 15 lb/A. We believe BEPAS18 burr medic will reduce overall costs of producing forage for livestock and wildlife while improving performance. It will reduce or eliminate the need for N-fertilizer on perennial warm-season grass pastures. An exclusive release is anticipated this fall.
We are also evaluating other annual *Medicago* species that have become naturalized in Texas, for use in other ecological zones of Texas.

Getting annual medics to the market: The good news and the bad news.

D. Oien
Timeless Seeds, Conrad, MT

Scientific research and on-farm experiences indicate that the use of annual medics is adaptable to the Northern Great Plains of North America. From a marketing standpoint, however, attempts to introduce annual medics under a variation of the classic Australian ley farming system have been a near failure. Identifying the barriers to acceptability in the marketplace may help direct future research in ways that will increase the adoption of medic farming systems by farmers.

Ten years of marketing medics have allowed the identification of the following barriers:

I. Paradigm Barriers.

Commonly held assumptions by Northern Plains cereal grain farmers that hinder the consideration of medics as a viable component in their farming system include:

A. Any broad leaf specie that regenerates is considered a weed by definition.

B. Control of natural processes outweighs the potential benefits of the natural processes themselves.

C. Soil moisture conservation results only from eliminating all plant growth during fallow periods.

D. Companion cropping or inter cropping is not economically viable under any circumstances.

E. Spring seeding season ends on a specific date (which varies by region), and a late seeding of annual medics or other cover crop would be doomed to failure.

II. Institutional Barriers.

Farmers who do desire to include annual medics in their farming systems face barriers from the institutions on which they depend. These constraints include:

A. In the past, federal farm programs punished any deviation from prescribed cropping patterns.

B. Crop insurance carriers reduce coverage for continuous cropping systems, and generally consider cover crops as continuous crops.

C. Commercial lenders are reluctant to finance innovative farming systems.

D. As a convenient and legitimate information source, Agricultural Extension Service often lags behind both land grant, private, and on-farm research.

III. Agronomic Barriers.

Many production and management questions remain unanswered for farmers.

The following areas deserve attention by the research community:

A. Variety development to account for agroclimatic zones.

B. More reliable stand establishment techniques.

C. Mechanical seed harvest techniques.

D. Allelopathic and other weed suppressing characteristics both during the medic years and for the ensuing crop.

E. Cultural, mechanical, grazing, and chemical pest management strategies for medic systems.

F. Use of annual medics in organic farming systems and direct seed/no till systems.

Summary.

Medics clearly have a place in the farming systems of the Northern Great Plains of North America. The wide scale adoption of such systems face numerous barriers, which need to be addressed by both the research community and the seed industry.

Segregational Vs. Mutational Genetic Loads as Sources of Inbreeding Depression in the Autotetraploid

Robin W. Groose
Plant Sciences Dept., Univ. of Wyoming, PO Box 3354, Laramie WY 82071 USA groose@uwyo.edu

Autotetraploid (2n=4x=32) alfalfa (*Medicago sativa* L.) suffers severe inbreeding depression upon repeated self-fertilization and displays progressive heterosis with successive hybridization. Therefore, it may be said that alfalfa bears a heavy genetic load. Genetic load might depress varietal performance, especially in narrow-based synthetic cultivars, and perhaps also in modern highly-selected multiple pest resistant cultivars.

Three models have been proposed to explain breeding behavior in alfalfa according to three forms of gene action: **(1) DOMINANCE** where, at individual loci, favorable dominant alleles mask deleterious/lethal recessive alleles; **(2) OVERDOMINANCE** involving heterozygous advantage of multiple functional alleles at individual loci; and **(3) PSEUDO-OVERDOMINANCE** due to complementation of favorable dominant alleles at different individual loci in tight repulsion phase linkage in chromosome segments or "linkats." These three models correspond to **(1)** a **MUTATIONAL LOAD** where nonfunctional recessive alleles are exposed with selfing;

(2) a **SEGREGATIONAL LOAD** where positive interactions among multiple alleles are lost with selfing; and **(3)** another form of **SEGREGATIONAL LOAD** where, as complementation among linkats is lost with selfing, individual loci become homozygous for recessive alleles. **MODEL (1)** might be considered the simplest, but Busbice and Wilsie (1966) proposed **MODEL (2)** because they observed that inbreeding depression was much more severe in autotetraploid alfalfa than would be expected based on the theoretical loss of heterozygosity (and increase in **F**, the probability that two alleles are identical by descent) which proceeds much more slowly in the autotetraploid than in the diploid. In their model involving true overdominance, a rapid loss of diallelic interactions among multiple alleles in highly heterozygous genotypes explains severe inbreeding depression. Because it is difficult to explain the existence of numerous heterotic alleles at an individual locus, **MODEL (3)** was proposed as a more realistic alternative (reviewed by Bingham et al., 1994).

In this paper, using concepts of genetic load and fitness, I explicitly evaluate these three models. I show that, for one version of Busbice and Wilsie's **MODEL (2)** involving exactly four different alleles at a locus, a population bears a very heavy overt genetic load (average fitness less than half of maximum fitness) but that the subsequent exposure of covert genetic load with selfing could still explain rapid inbreeding depression. Similarly, the four locus/four linkat version of **MODEL (3)** illustrated by Bingham and co-workers with a single favorable dominant allele per linkat bears a seemingly impossible overt genetic load. However, reformulated, with each linkat bearing a single recessive lethal allele, an intermating population bears a much smaller overt load but exposes a large covert load with selfing. Finally, I show that **MODEL (1)** based on simple dominance may be a much better explanation for inbreeding depression in the autotetraploid than has previously been appreciated. This is because, despite the slower theoretical approach to homozygosity in the autotetraploid than in the diploid, the autotetraploid may bear a much larger mutational load. It is well known that in a diploid where dominance is complete, q, the equilibrium frequency of an unfavorable recessive allele in an intermating population depends on a balance between mutation and selection where the frequency of homozygous recessive genotypes (q^2) is equal to the ratio of the mutation rate (μ) to the selective disadvantage (s) of the recessive homozygote, i.e., $q^2=\mu/s$. I show that in the autotetraploid the frequency of the recessive homozygote is the same, i.e. $q^4 = \mu/s$. Then, for all loci where mutation rates and selective disadvantages are the same between ploidy levels, I demonstrate two important results. First, overt mutational loads are equal between ploidy levels, i.e. $q_{2x}^2 = q_{4x}^4 = \mu/s$. Second, total (and covert) loads are much larger in the autotetraploid because, by the same equation, the frequencies of recessive alleles are much higher in the autotetraploid.

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Cytogenetic Studies of the Nine Germplasm Sources of Alfalfa

Gary R. Bauchan[1] and M. Azhar. Hossain[2]
[1]USDA-ARS, Soybean & Alfalfa Research Lab. Beltsville, MD and [2]University of Maryland, Natural Resources and Landscape Architecture Department, College Park, MD

Karyotypic analysis was conducted utilizing a computerized image analysis system of C-banded chromosomes of the nine germplasm sources of tetraploid alfalfa (*Medicago sativa* L.)(Barnes et.al. 1977). These nine germplasm sources were designated by the Alfalfa Crop Germplasm Committee as the populations which represent most of the alfalfa germplasm that has been used to develop modern alfalfa varieties. The PI's which were used in this study are: 'Falcata' PI 536531, 'Ladak' PI 536532, 'Varia' PI 536533, 'Chilean' PI 536534, 'Peruvian' PI 536535, 'Indian' PI 536536, 'Turkistan' PI 536537, 'Flemish' PI 536538, and 'African' PI 536539. The seeds for this study were obtained from the National Plant Germplasm System in Pullman, WA. The C-banding technique and computerized image analysis system utilized in this study are described by Bauchan and Hossain (1997). Karyotypic analysis of tetraploid alfalfa revealed that alfalfa has four nearly identical sets of chromosomes based on their chromosome morphology and C banding patterns, thus providing support that alfalfa is an autotetraploid. C-banding polymorphisms were detected in the number, position and intensity of terminal and interstitial bands within a germplasm source. A wide range of differences were also observed between the nine germplasm sources. The 'Falcata's source is strikingly different from other germplasm sources due to a fewer number of terminal and interstitial bands. 'Falcata' chromosomes have primarily C-bands at their centromere. The 'African' germplasm has the largest number of C-bands thus far studied with all of the chromosomes having centromeric bands, and in addition, all of the chromosomes have telomeric bands on their short arms. Except for chromosome 7, all of the chromosomes have interstitial bands on their short arms and chromosomes 1, 2 and 3 each have one prominent interstitial band on their long arms. The ranking of germplasm sources from greatest number of C-bands to least number of bands studied thus far is as follows:'African'>'Peruvian'>'Chilean'>'Flemish>'Indian'>'Varia '>'Ladak'>'Turkistan'>'Falcata'. It is interesting to note that the more winter hardy germplasm sources have fewer C-bands indicating the incorporation of 'Falcata' germplasm in alfalfa.

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Measuring Genetic Distances in the Alfalfa Core Collection

D. Z. Skinner
USDA-ARS and Agronomy Dept., Kansas State University, Manhattan, KS

Most of the chloroplast genome in higher plants is highly conserved. However, two hypervariable regions have been identified in the alfalfa (*Medicago sativa* L) chloroplast genome. The hypervariable regions are being used as a means to assess the degree of relationship among the accessions of the perennial alfalfa core collection. Genetic distance was measured as the proportion of hypervariable fragments not in common among two plant accessions; 24 plants are assayed from each accession.

The country of origin of each PI was determined from the USDA Germplasm Resources Information Network (GRIN) system (http://www.ars-grin.gov). The average distance of each accession to all other accessions was calculated and used to calculate average between-country-of-origin distances (Table 1). The likely basic germplasm source (Barnes *et al.*, 1977) of each PI was determined from the country of origin and its position on the basic germplasm source map. The average genetic distances of each of the basic germplasm sources to all other germplasm sources are shown in Table 1a.

Mean genetic distances ranged from 0.33 to 0.99 with a mean of 0.46. A larger average genetic distance was interpreted to indicate a more distinctive population, *i.e.*the PIs from that group (country or germplasm source) were most unlike the other PIs. By this criterion, the most distinctive PIs were of the African and Chilean germplasm sources (Table 2). Interestingly, the most distinctive PIs grouped by country of origin tended to be from countries which are not considered to be part of the basic germplasm origins, such as China and parts of the Great Plains of the US (Table 1). These results indicate that the hypervariable regions of the chloroplast DNA provide a means to distinguish accessions and populations, and to provide a quantitative measure of relatedness.

Table 1. Countries of origin or germplasm sources and mean genetic distance of PIs from

Country	No.	Mean distance	Country	No.	Mean distance	Country	No.	Mean
Afghanistan	4	0.42	Lithuania	1	0.35	Uzbekistan	5	0.41
Algeria	4	0.41	Morocco	14	0.48	Venezuela	1	0.45
Argentina	4	0.61	Pakistan	2	0.42	United States		
Armenia	1	0.48	Peru	9	0.37	Alaska	1	0.44
Australia	2	0.45	Poland	1	0.43	Montana	7	0.38
Bolivia	5	0.36	Romania	4	0.39	Iowa	1	0.73
Canada	14	0.46	Russian	4	0.42	Nebraska	5	0.39
Chile	1	0.37	Saudi Arabia	1	0.36	N.Dakota	1	0.54
China	8	0.70	Siberia	1	0.35	S. Dakota	5	0.43
Cyprus	1	0.53	South Africa	1	0.37	Table 1a Basic origins		
Czechoslovakia	1	0.49	Soviet Union	21	0.46	African	6	0.52
Ecuador	1	0.38	Spain	4	0.64	Chilean	5	0.48
Egypt	4	0.58	Sweden	2	0.39	Falcata	4	0.45
England	1	0.39	Tajikistan	1	0.39	Flemish	4	0.39
Estonia	1	0.34	Turkey	27	0.43	Indian	1	0.44
France	4	0.39	Ukraine	4	0.46	Ladak	1	0.42
Greece	1	0.46	Iraq	1	0.36	Peruvian	9	0.37
India	6	0.44	Jordan	1	0.87	Turkistan	5	0.47
Iran	3	0.60	Kazakhstan	4	0.48	Varia	7	0.41

Reference

Barnes, DK, Bingham, ET, Murphy, RP, Hunt, OJ, Beard, DF, Skrdla WH, and Teuber, LR. 1977. Alfalfa Germplasm in the United States: Genetic Vulnerability, Use, Improvement and Maintenance. U.S. Department of Agriculture, U.S. Printing Office. Technical Bulletin No. 1571.

Self-Incompatibility Research at the Beltsville Agricultural Research Center

T. A. Campbell[1], G.R. Bauchan[1], Z.L. Xia[1] and J.L. Hansen[2].
[1]USDA-ARS, Soybean and Alfalfa Research Laboratory, Beltsville MD 20705. [2]Department of Plant Breeding and Biometry, Cornell University, Ithaca, New York 14853.

High levels of inbreeding depression and evidence of heterosis led to early consideration of the development of hybrid alfalfa, however, it was concluded that self-incompatibility in alfalfa was not stable enough for the commercial production of hybrid seed and a cytoplasmic male sterility system was advocated. A major advantage to the use of a self-incompatibility system for producing hybrid alfalfa seed is that parental clones could be randomized in the field, and that F1 seed could be harvested from all plants. Difficulties inherent in establishing a small number of genotypes for hybrid seed production may be alleviated through the field establishment of somatic embryos mass-produced in a bioreactor. Such a hybrid seed system may not be economically viable, however, a system in which a synthetic generation was produced from the F1 progenies could have some potential. We conducted several experiments designed to assess the potential of a self-incompatibility system for enhancing hybridity and heterosis in alfalfa.

Our first experiments studied the responses of self-incompatible clones to four temperature regimes and revealed that clones with stable self-incompatibility and excellent male and female fertility could be selected. A factorial analysis of genetic variation using parents which represented a broad range of self-compatibility demonstrated that self-incompatibility was heritable (narrow-sense heritability=0.28) and that additive genetic variation was most important. In a later experiment, we evaluated the effects of self-incompatibility on forage yield in four F1 crosses and four F1/Syn1 pairs (Test 1), and in two partial diallel crosses within stable self-incompatible (SI) and self-compatible (SC) clones (Test 2). In Test 1, yields of some F1 crosses and Syn1 populations were significantly greater than those of the check 'Saranac AR.' Except for one case, F1 yields were significantly larger or smaller than Syn1 yields for each F1/Syn1 pair. These differences may be due to variations in hybridity traceable to variations in stability of the self-incompatibility trait and/or to progressive heterosis in some Syn1 generations. In Test 2, some of the F1 yields were significantly larger than those for the checks 'KSNYPx' and 'Oneida VR.' Most importantly, yields of the SI crosses were superior to those of the SC crosses, most likely due to reduced inbreeding depression in the SI diallel. RAPD and Anchored Microsatellite Priming analyses revealed no evidence of a relationship between self-incompatibility and inbreeding. We concluded that SI clones could be the products of inbreeding and/or a heritable, self-incompatibility mechanism. The importance of each factor would depend on genetic distances (GD's) among the clones.

Environmentally stable SI clones separated by large GD's would be a good basis for a SI-based hybrid alfalfa system or for use in other breeding schemes designed to minimize inbreeding while maximizing heterosis. Yield improvements of the F1 crosses and Syn1 populations over checks were not sufficient to justify the added expense of implementing the proposed F1/Syn1 seed production system. However, this system may have more potential if based on SI clones selected for maximum genetic distance and specific combining ability from more diverse populations with superior disease resistance than those studied here.

Risk of Alfalfa Transgene Dissemination and Scale Dependent Effects

Paul C. St. Amand[1], Daniel Z. Skinner[2], and Richard N. Peaden[3]
[1]Kansas State University and [2]USDA/ARS Agronomy Department, 2004 Throckmorton Hall, Manhattan KS 66506, and [3]USDA/ARS, Irrigated Agricultural Research Center, Prosser WA 99350.

Pollen can function as a vehicle to disseminate introduced, genetically-engineered genes throughout a plant population or into a related species. The measurement of the risk of inadvertent dispersal of engineered genes must include an assessment of accidental dispersion of pollen. Factors to be considered include the rate of pollen spread, the maximal dispersion distance of pollen, and the spatial dynamics of pollen movement within seed production fields; none of which are known for alfalfa (*Medicago sativa* L.), an insect-pollinated crop species. Using a rare, naturally-occurring molecular marker, pollen dispersal can be tracked without introducing engineered genes into the environment. In this study, a suitable marker was found, using PCR-based methods, in an intron of the alfalfa glutamine synthetase gene. This marker system was used to track pollen movement within and away from seed-production fields; random amplified polymorphic DNA (RAPD) fragments were used to detect cross-pollination events in widely-dispersed alfalfa plants escaped from cultivation. Results indicated that leafcutter bees (*Megachile* spp.) used in commercial seed production show a bi-directional bias when pollinating, primarily resulting in the movement of pollen from marker plants directly toward and away from the bee domicile. Within-field pollen movement was detected only over distances of 4 m or less. Long-range dispersal of pollen from alfalfa hay and seed production fields has been confirmed for distances up to 1000 m using small trap plantings of alfalfa (Fig. 1). Individual plants grown within urban areas at least 800 m from known alfalfa plants failed to produce seeds. An assessment of scale dependencies is lacking from most risk assessment studies. A novel field design and marker system was developed to allow an accurate investigation of scale dependencies on gene movement by comparing dispersal from research scale plots with that of commercial scale fields. In this study, pollen moved from commercial scale fields three times farther than from small, research scale fields. Data suggest that complete containment of transgenes within alfalfa seed or hay production fields would be highly unlikely using current production practices.

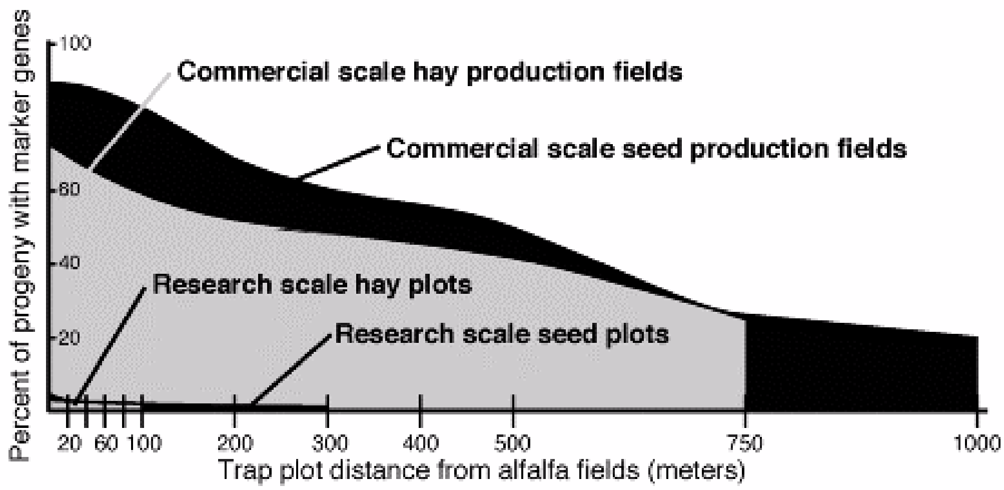

Figure 1. Marker gene movement away from commercial and research scale alfalfa seed and hay production fields.

Cold Acclimation-induced Changes in Gene Expression in Roots and Crown Buds

of Alfalfa Germplasms Selected for Contrasting Fall Dormancy

S.M. Cunningham[1], J.A. Gana[1], L.R. Teuber[2], and J.J. Volenec[1]
[1]Dept. of Agronomy, Purdue University, West Lafayette, IN 47907-1150, and [2]Dept. of Agronomy and Range Science, University of California, Davis, CA 95616-8515

Alfalfa improvement programs would benefit by understanding mechanisms controlling fall dormancy and winter survival. Our objectives are to identify and characterize genes associated with fall dormancy reaction and winter survival of alfalfa. Selections from ëCUF-101í (fall nondormant) for greater fall dormancy produced plants with reduced herbage growth in autumn and markedly improved winterhardiness (Table 1).

Table 1. The influence of selection for contrasting fall dormancy on plant height in fall and winter survival. Note the large increase in winter survival of CUF 101 selected for greater fall dormancy (CUF 101-L, shaded data) (Cunningham et al., Crop Sci. 38: in press).

Cultivar	Selection Direction	Shoot Hgt in Fall, cm	Winter Surv., %
Norseman-L	Greater Fall Dormancy	14	100
Norseman-O	Original Cultivar	19	98
Norseman-H	Less Fall Dormancy	35	93
CUF 101-L	Greater Fall Dormancy	36	93
CUF 101-O	Original Cultivar	50	1
CUF 101-H	Less Fall Dormancy	51	7

We used differential display to analyze root and bud tissues for transcript differences between CUF 101 and CUF 101-L, the population selected for increased fall dormancy. Northern and dot-blot analyses to date have confirmed cold acclimation-responsive (*CAR*) cDNA clones that are differentially expressed between CUF 101 and CUF 101-L. The mRNA levels for certain *CAR* cDNAs were about 10-fold more abundant in December in CUF 101-L which survived winter when compared to CUF 101 (Fig 1).

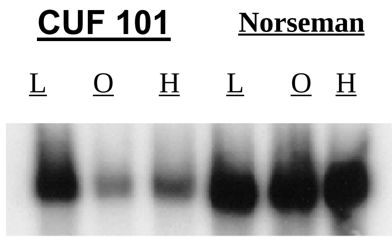

Figure 1. Northern analysis showing differential expression of *CAR* genes in alfalfa roots in December. See Table 1 for germplasm descriptions.

Nucleotide sequence analyses of two of our *CAR* cDNA clones showed high homology to previous identified cold-acclimation-specific cDNAs (*cas*15A and *cas*15B) from alfalfa (Laberge et al., 1993; Monroy et al., 1993). Our results suggest we have cloned new members of the *cas*15 gene family that are highly expressed during hardening of fall dormant alfalfa. Expression of these *CAR* genes is positively associated with winter survival. We have cloned full-length cDNAs for several of our *CAR* genes. We are currently analyzing the deduced amino acid sequences for to elucidate their function. We plan to transform the nonhardy CUF 101 and other nondormant cultivars with constructs containing these *CAR* genes to determine their impact on winter survival and fall dormancy.

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Evaluating Salt Tolerance: Some Experiences with Nondormant Alfalfa.

Steve Smith
Department of Plant Sciences, University of Arizona, Tucson, AZ 85721

Without elaborate irrigation or drainage systems, accumulation of excessive levels of soil salinity (salinization) is inevitable in most environments where irrigated alfalfa is grown. Worldwide, nondormant alfalfas are often grown in warm desert environments where irrigation is essential and the potential for salinization is high. Alfalfa is considered moderately sensitive to salinity and yield reductions of about 15% will result with electrical conductivity (EC) in the soil solution of only 4 dS m^{-1}. EC's of soil solutions on over 40% of the cropland in the Imperial Valley of California average above 4 dS m^{-1}. Changes in cropping practices or improvement of crop salt tolerance through plant breeding may offer temporary solutions to salinization problems in these environments.

Breeding for improved salt tolerance in alfalfa is complicated by a variety of factors, most notably the very low heritability of tolerance. Different growth stages in alfalfa also appear to respond independently to salinity. Mass selection at increasing levels of NaCl has been successful in producing a population that germinates in very saline environments (35 dS m^{-1}). Populations with less germination salt tolerance have been produced by commercial plant breeders. Describing germination salt tolerance has been problematical. Using data from trials conducted using a range of salinities, we have shown that probit analysis permits calculation of the level of salinity where a certain proportion of seeds in a sample (e.g., 50%) would be expected to germinate.

We have developed and refined a greenhouse procedure to evaluate forage yield of single plants under moderate salt stress. This technique involves plants grown in 23-cm conetainers in artificial soil. They are irrigated with water having EC=7.3 (or 9.1) dS m^{-1}, and forage is harvested and weighed three times. By growing populations under these conditions but using non-saline irrigation it is possible to measure salt tolerance per se by the saline/non saline yield ratio. Mass selection using this protocol has resulted in populations with increased forage yield under these experimental conditions. Some populations produced using other selection procedures exhibit both increased yields and salt tolerance. Selection using more saline irrigation water (10 dS m^{-1}) may be worthwhile. Relationships between performance in this greenhouse trial and in the field are not well known. While economical, visual selection appears unlikely to be as efficient as the protocol outlined above for increasing either forage yield with saline irrigation or salt tolerance.

Mechanism(s) of tolerance to salinity utilized by alfalfa are unknown. A better understanding of tolerance mechanisms could permit more deterministic approaches to plant breeding. However, this appears unlikely in the near future.

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Check Cultivars, Locations, and Management of Fall Dormancy Evaluation

L.R. Teuber, K.L. Taggard, L.K. Gibbs, S.E. Orloff, S.C. Mueller, C.A. Frate, D.H. Putnam and J.J. Volenec
Agronomy and Range Sci. and UC Cooperative Extension, Univ. of California; Department of Agronomy, Purdue University

It has become increasingly clear that all cultivars do not respond equally in the fall to changes in photoperiod and temperature. Additionally, some "old" check cultivars produce inconsistent data from year to year and location to location. Lahontan has been particularly susceptible to this criticism. Yet, fall dormancy class (FD) is frequently assigned based on a single trial. Evaluation of fall dormancy in single environments has led to misclassification of some cultivars. To alleviate some of these problems, a "new" larger set of check cultivars was proposed in 1995 with three cultivars for each fall dormancy class. However, inclusion of these checks was largely based on data produced at a single Midwest location. We were concerned that multiple (but not the same) checks grown across a broad range of environments (latitudes and years) could result in further confusion when assigning fall dormancy class. To elucidate the magnitude of this problem, we initiated studies in 1995 to evaluate fall dormancy using four California locations differing substantially in latitude and temperature. Additionally, we wished to identify appropriate check cultivars for extremely non-dormant germplasm (Class 10 and 11). Trials at each location were conducted according to the standard protocol for evaluating fall dormancy and included all the old and new check cultivars (1). Fall regrowth scores were square root transformed to remove heterogeneity of variance creating a variable, natural plant height (NPH). We also utilized the data to determine variance components and the theoretical standard errors for NPH of a cultivar mean. Significant GxE interactions are present for NPH when all cultivars are included in an ANOVA. The G*L*Y interaction is 6x the G*Y and 50x the G*L variance. Rank changes among the checks result in some "check" cultivars ordering with cultivars representing dormancy groups as much as two classes from their proposed classification. A sub-set of the check cultivars has been identified -- Maverick, 1; 526, 2; 5246, 3; Legend, 4; Archer, 5; ABI 700, 6; Dona Ana, 7; Pierce, 8; CUF 101, 9; UC-1887, 10; and UC-1465, 11. When the NPH of this sub-set is regressed against FD the same slope is equivlivent to that of old checks and there is minimal deviation from regression (FD= 6.36(NPH) ó 7.68, r^2=0.992). We detected no significant GxE among these cultivars. Finally, because of the GxE among all cultivars, FD should be assigned based on two years of testing at a minimum of three locations.

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The Relationship Between Bacterial Wilt Resistance and Persistence is Not the Same Among Alfalfa Varieties

Edwin T. Bingham
Agronomy Department University of Wisconsin, Madison, WI 53706

Experimental Synthetics produced from four year old field survivors of 'Vernal', 'Wrangler', and 'Oneida' alfalfa were 8-12% higher in bacterial wilt resistance than the respective original varieties. This result is consistent with natural selection that has been important in the history of alfalfa production (3). However, experimental svnthetics produced from field survivors of 'Arrow' and 'Elevation' dug from the same nursery (1) decreased 22 and 8%, respectively in bacterial wilt resistance. This was unexpected, and appears to be a lead in understanding the persistence problem (2,4). It appears that some of the bacterial wilt resistant plants in Arrow and Elevation lacked persistence and were eliminated in the first three years. Many of the survivors in Arrow and Elevation at four years were escapes from bacterial wilt. This set the stage for elimination of plants susceptible to bacterial wilt and may be one of the reasons for the rapid stand loss in these varieties. The relationship between bacterial wilt resistance and persistence is being studied in additional varieties. This research and research aimed at gaining some insight into the genetics of persistence per se, will be described.

Previously, a study of 30 years of Wisconsin alfalfa variety trials revealed that correlations of disease resistance with yield decreased over time indicating that more resistant varieties on average were less persistent (2). An independent study using data from eight Mid west states also found a small and sometimes negative impact of disease resistance ratings on alfalfa productivity in the third year (4). Our goal as we enter the next century is balanced improvement of yield, persistence, and pest resistance.

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Maturity and plant density effects on stem and leaf traits for alfalfa biomass production

JoAnn F. S. Lamb, Craig C. Sheaffer, Deborah A. Samac, and Neal P. Martin.
USDA-ARS, Plant Science Research Unit, and University of Minnesota, St. Paul, MN

Alfalfa was chosen as the dedicated biomass feed stock resource for a 75 MW power station at Granite Falls, MN (De Long, et al., 1995). In this biomass production system alfalfa hay will be fractionated into stem material for conversion to electricity and leaf meal to be sold as a high quality livestock protein supplement. To meet the economic demands of this new biomass production system the yield of both leaf and stem fractions will need to be maximized. A study was established to assess environmental and harvest management effects on biomass production in alfalfa. Five unrelated germplasms, two Flemish sources, (Barnes et al. 1977) and three moderately dormant North American sources were established at two locations at four plant population densities (450, 180, 50, and 16 plants m-2). All germplasm by plant density combinations were harvested at two stages of plant maturity, early bud (3 harvests per season) and late flower/early pod (2 harvests per season). Stem and leaf yield, percent crude protein (%CP) and percent neutral detergent fiber (%NDF) are being compared among germplasms under the different plant density and maturity treatment combinations. Desirable stem traits for conversion to electricity include high yield, high % NDF and low % CP. Preliminary data from one year and location showed the greatest seasonal leaf and stem yield for all germplasms was found at the 180 plants m-2 density at the late flower/early pod maturity under a 2 harvest management. No differences in stem % CP was found among the alfalfa germplasms. Stems harvested at the late flower/early pod maturity had lower % CP and greater % NDF than those harvested at early bud and the 450 plants m-2 density had significantly greater stem % CP and lower % NDF compared the rest of the plant density treatments. Leaf % CP was greater under early bud management treatment, but season total harvested leaf crude protein was the same when harvested at either the early bud (3 cuts) or the late flower/ early pod (2 cuts) maturity stages. The three moderately dormant North American germplasms sources had greater leaf yield than the Flemish types, but the Flemish types had fewer lodged plants. Preliminary results demonstrated that decreased plant population densities and harvesting forage at later maturities may enhance the profitability of an alfalfa biomass production sytem.

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Agronomic and morphological diversity of alfalfa germplasm in Mexico.

J.J. Marquez-Ortiz[1], G. Nunez-Hernandez[1], and S. Salinas-Carmona[2]
[1]C.E. La Laguna/INIFAP, Apdo. Postal 247, CP 27000 Torreón, Coah., Mexico, [2]Semillas Berentsen, Carr. Panamericana Km. 285, CP 38000 Celaya, Gto. Mexico.

Alfalfa was introduced from Spain to Mexico almost 500 years ago. Barnes et al. (1977), suggested that alfalfa germplasm adapted to Mexico could belong to the Chilean group. Mexican alfalfa landraces have shown good forage yields, persistence, and resistance to specific diseases in their areas of adaptation. The objective of this study was to characterize, compare, and classify alfalfa germplasm available in Mexico according to agronomic and morphological traits.

Forty-one alfalfa genotypes (25 landraces and 16 commercial cultivars) were seeded 1 December 1995 at Matamoros, Coahuila, Mexico under a randomized complete block design with four replications. Plots were 20-cm diameter microplots separated at 75 cm. Above-ground morphological variables measured in 1996 were: stem basal diameter; number of top and bottom internodes per stem; and bottom and top central leaflet width and length. Agronomic variables were: spring, summer, fall and annual average dry matter yield, plant height, and leaf:stem ratio. The variables were analyzed by variance, correlation, principal components, and average linkage cluster analyses to determine differences among genotypes, select classification variables, and determine groups based on similarities among genotypes.

Genotypes had significant effects ($P<0.05$) on spring, summer, fall, and annual average dry matter yield and plant height, top and bottom internode length, top central leaflet width, top and bottom average leaflet width and leaf:stem ratio. Classification variables used for principal component and cluster analyses were annual dry matter yield, fall plant height, top and bottom internode length, average leaflet width and leaf:stem ratio. Cluster analysis classified genotypes into five germplasm groups: DK-189, Oaxaca, Commercial, Tanhuato, and San Miguelito. In general, these groups coincided with their area of adaptation. Each group showed at least one trait which made them different from the other groups: DK-189=highest leaf:stem ratio, Oaxaca=lowest average annual yield, Commercial=highest yield and internode length, Tanhuato=highest fall plant height, and San Miguelito=intermediate for most traits. These results suggest the presence of significant variation among alfalfa genotypes available in Mexico and that such variation can be classified, at the moment, into five different germplasm groups.

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Improving Alfalfa Germination and Vigor Under Early Spring Conditions

Kathy L. E. Klos and E. Charles Brummer
Iowa State University, Ames, IA 50011

No-till seeding of alfalfa into existing pasture is often complicated by competition with weeds and grasses. The need for herbicide application may delay planting past the optimum date, and increase problems due to disease. Alfalfa seedlings exhibiting early germination and rapid growth in cold (above freezing) soils may be able to establish more successfully in the early spring, reducing problems with disease and weed competition. Previous studies have identified variation in germination and growth response curves to different temperatures among forage legumes (Brar et al., 1990, 1991); and differences in rate of germination and initial radicle growth rate among and within alfalfa cultivars (Esvelt and Brummer, 1996; Klos and Brummer, 1997). The purpose of this study was to evaluate four methods of recurrent mass selection for the improvement of alfalfa seedling establishment and growth under early spring conditions. Two to three cycles of mass selection were conducted within six commercial alfalfa cultivars. Individuals were selected based on days to germination in germination boxes at 5 oC, seedling growth in a 10 oC growth chamber, and for extremes of germination rate and seedling growth rate under these conditions combined. Resulting lines and commercial cultivar source populations were planted in the early spring at two locations in Iowa, and evaluated for emergence eight days after planting, seedling height one month after planting, dry matter yield, and other plant characteristics. Lines were also evaluated for germination and seedling growth under selection conditions. This paper will present results of the evaluation of selected lines for field emergence and seedling growth, and correlations between field emergence, seedling height in the field, and traits measured under laboratory conditions. Methods of selection for seedling establishment will be compared within and among commercial cultivar source populations.

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Resistance to *Sclerotium rolfsii* in Populations of Alfalfa Selected for Resistance to *Sclerotinia trifoliorum*

Robert G. Pratt and Dennis E. Rowe
USDA, ARS, Waste Management & Forage Research Unit, Miss. State, MS 39762

Sclerotium rolfsii causes diseases that are usually referred to as "Southern blight" on a wide range of crops in the southeastern USA. Southern blight occurs during warm summer months, is favored by high humidity, and causes rapid collapse and death of infected plants. These often bear external white mycelium and numerous small, yellow to brown sclerotia of *S. rolfsii*. Among forage crops in the Southeast, Southern blight is especially damaging on white clover, but it also causes significant losses on alfalfa.

Sclerotium rolfsii and *Sclerotinia trifoliorum* are taxonomically unrelated fungal pathogens that cause disease under very different environmental conditions. Nevertheless, these two fungi bear some similarities in modes and mechanisms of pathogenesis. Both cause rapid rotting of leaves, petioles and stems that often results in death of infected plants. The mechanism of pathogenesis by both fungi is believed to involve the secretion of oxalic acid, which weakens or destroys host cells in advance of hyphal growth. From these similarities in parasitism, it appeared that resistance to *S. trifoliorum* and *S. rolfsii* in alfalfa might be related. Therefore, this study was conducted to determine whether populations of alfalfa selected for resistance to *S. trifoliorum* also manifest resistance to *S. rolfsii*.

Evaluations were performed on two alfalfa populations previously developed for resistance to *S. trifoliorum* (STR and MSR) and on four new populations. Most evaluations of resistance to *S. rolfsii* were performed by a leaf tissue assay similar to that developed for *S. trifoliorum*. Whole plant inoculations also were performed to verify resistance in STR and MSR. In repeated leaf-tissue assays of STR and MSR, both populations expressed resistance to *S. rolfsii* in comparison to the parent cultivar, Delta. In whole-plant inoculation experiments with *S. rolfsii* and plants of different ages, where resistance was evaluated by percentage plant survival, both MSR and STR had greater survival than Delta in repeated experiments. However, although MSR is more resistant to *S. trifoliorum* than is STR, it was suggested from results of these and other experiments that STR may be more resistant to *S. rolfsii* than is MSR.

Four additional populations with resistance to *S. trifoliorum* were developed from the cultivars Magnum-III Wet, WAMPR, WL-323, and 5472 by selection with leaf inoculation techniques and intercrossing. Random populations were developed from the same cultivars by selecting similar numbers of plants at random. Resistance to *S. trifoliorum* in the populations selected for resistance, in comparison to parent cultivars and random populations, was demonstrated by leaf assays and by whole plant inoculations. In repeated leaf-tissue assays, all four new populations developed for resistance to *S. trifoliorum* also expressed resistance to *S. rolfsii* in comparison to the parent cultivars, random populations, or both.

From these results, it is concluded that selection for resistance to *S. trifoliorum* in alfalfa also confers increased resistance to *S. rolfsii*. Therefore, populations selected for resistance to *S. trifoliorum* during winter and early spring might also demonstrate improved persistence during summer months in the southeastern USA because of increased resistance to *S. rolfsii*.

Development of a Standardized Test for Evaluating Alfalfa for Resistance to *Aphanomyces euteiches* Race 2

B. D. Hudelson and C. R. Grau
University of Wisconsin-Madison, Madison, WI 53706

Aphanomyces euteiches causes seedling and root health problems of alfalfa especially when soil moisture is excessively high. WAPH-1 alfalfa germplasm was released in 1992 and was intended as a source of resistance and a resistant check for the alfalfa industry (1). Isolates highly virulent to WAPH-1 were discovered in 1988 and were designated as race 2. Isolates with low virulence to WAPH-1 were designated as race 1. The importance and impact of race 2 has not been documented, but some level of anticipatory breeding is recommended to alfalfa breeders. Thus, a standardized test for *A. euteiches* race 2 has been developed. WAPH-5 alfalfa germplasm was developed as a resistant standard for race 2 isolates of *A. euteiches.* Initially, attempts were made to improve WAPH-1 for resistance to race 2 isolates, but genetic gain could not be made after three cycles of selection. Fifty plant introductions (PIs) were identified that expressed variability for resistance to race 2 isolates. Although several PIs were possible candidates, PI 468018, PI 439006, and PI 468471 were used to incorporate resistance to race 2 into an adapted germplasm pool (ADP) derived from WAPH-1. Plants of each plant introductions were challenged with race 2 isolates (NC-1 and WI-98) of *A. euteiches.* Race 2 resistant phenotypes were selected and intermated with ADP plants which had been previously selected for resistance to *Phytophthora medicaginis*, *Verticillium albo-atrum* and *Colletotrichum trifolii.* A base population was kept separate for each PI source for three cycles of selection. Resistant plants from each PI source were selected in cycle 4 and intermated to develop WAPH-3. Two additional cycles of selection were performed to develop WAPH-5. The development of WAPH-5 and the standardized test was done in collaboration with private sector alfalfa scientists from ABI Alfalfa, FFR Cooperative, Cal-West, Forage Genetics, W-L Research, Pioneer and Dairyland.

Table 1. Reaction of alfalfa populations to isolates of race 1 and race 2 of *Aphanomyces euteiches*

Population	Isolate MF-1 (race 1)			Isolate NC-1 (race 2)		
	% Res. Plants	Transformed Res. Plants	DSI	% Res. Plants	Transformed Res. Plants	DSI
Agate	8	0.23	3.48	3	0.15	3.84
Saranac	3	0.12	3.65	2	0.07	3.96
WAPH-1	44	0.72	2.58	6	0.20	3.66
WAPH-3	54	0.83	2.30	26	0.50	3.00
WAPH-4	71	1.03	1.88	44	0.72	2.62
WAPH-5	71	1.03	2.02	50	0.79	2.56
LSD (0.05)	---	0.13	0.30	---	0.17	0.35
CV%	---	18.6	9.8	---	38.2	8.2

Reference

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Improved Seedling Health, Yield, and Stand Persistence With Aphanomyces-Resistant Alfalfa Following Natural Epidemics

Paul Vincelli and Jimmy Henning
Departments of Plant Pathology and Agronomy University of Kentucky, Lexington, KY 40546-0091

Although alfalfa-infecting strains of *Aphanomyces euteiches* are widespread in Kentucky soils, clear proof that alfalfa performance could be improved using varieties resistant to Aphanomyces root rot (ARR) was lacking. Epidemics of ARR in two recent alfalfa variety trials with entries ranging from susceptible to highly resistant to ARR allowed evaluation of the effect of such resistance on plant performance.

At the Eden Shale trial (seeded Apr 96; severe pressure from ARR), such variables as seedling health, alfalfa yield, and stand vigor during the seeding year were all significantly ($P<0.05$) improved by using ARR-resistant varieties. In 1997, alfalfa yield and stand vigor were also substantially better in varieties resistant to both ARR and Phytophthora root rot (PRR) than in varieties resistant to PRR alone, demonstrating value to ARR resistance past the seeding year. At the UKREC trial (seeded Apr 97, moderate pressure from ARR), while seedling health was improved significantly during the seeding year, yields in 1997 were not correlated to percent ARR resistance. Likewise, stand survival through the winter was substantially poorer only in varieties susceptible to PRR. We concluded that, under conditions highly conducive for the disease, Kentucky producers can expect a substantial improvement in seedling health by using ARR-resistant alfalfa varieties. Varieties with ARR resistance ratings of R or HR are expected to consistently outperform susceptible varieties under severe disease pressure, and under some environmental conditions, may do so under moderate disease pressure, as well.

Although our data regarding the agronomic value of ARR-resistant varieties were convincing, we documented sources of variation in plant health which also merit discussion. For example, varieties with a similar ARR resistance rating did not perform equally with respect to various measures of plant health following a severe outbreak of ARR; in simple terms, alfalfa varieties with the same ARR resistance rating are not created equal. While this result is not unexpected, it is significant and warrants affirmation. Another, less intuitive, result was that, under severe ARR pressure, varieties exhibiting improved plant health and performance also exhibited greater variation in plant health and performance. In the Eden Shale trial, variances for seedling health, alfalfa yield, stand vigor, and stand density increased greatly as the means for these variables increased. This pattern was not observed in the UKREC trial, where disease pressure was moderate. This result is important, because it suggests that, under high disease pressure, performance of the currently available ARR-resistant varieties may vary considerably within or among fields. This result is consistent with our own observations in commercial fields, in that we have documented fields planted to alfalfa varieties highly resistant to ARR with rather serious cases of the disease. The reason for this variation is being investigated.

Blossom blight of alfalfa: Distribution in western Canada and impact on seed yield

B.D. Gossen[1], J.D. Holley[2], L.M. Harrison[3] and S.R. Smith[4] [1]AAFC Research Centre, Saskatoon, SK, Canada, S7N 0X2; [2]Crop Diversification Centre, Brooks, AB, T0J 0J0; [3] Alberta Agriculture, Fairview, AB, T0H 1L0; [4]Dep. Plant Science, University of Manitoba, Winnipeg, MB, R3T 2N2.

Epidemics of blossom blight [*Botrytis cinerea, Sclerotinia sclerotiorum*] on alfalfa occurred across the Canadian prairies in 1993, in scattered areas in 1994, and in northern areas of Alberta and Saskatchewan in 1995 - 96. In 1997, severe drought prevailed across the region during flowering, but there were a few areas that received timely showers and they had moderate amounts of blossom blight. In 1995 to 1997, surveys were carried out across the region to assess disease severity; grower-cooperators plated flowers onto agar media at regular intervals during flowering and sent them to a central laboratory for assessment. *Botrytis cinerea* was the most common pathogen at most sites, but *S. sclerotiorum* predominated at several sites in southern Alberta. Blossom blight epidemics were consistently associated with prolonged cool, wet weather during flowering. Seed production under wet conditions is affected by reduced flower production and reduced pollinator activity, as well as by disease. As a result, the inconspicuous symptoms associated with moderate levels of blossom blight infection are easily overlooked. A test kit, designed to assist growers in assessing levels of infection in their fields, is being assessed by a limited number of growers in 1998, and should be available for general use in 1999. To determine the impact of blossom blight on seed yield, fungicide application during flowering was examined at 9 sites across western Canada in 1995, 13 sites in 1996, and 8 sites in 1997. Where blossom blight was severe, and levels stayed high for several weeks, seed yield in the controls was very low (occasionally less than 100 kg/ha). Application of benomyl consistently reduced flower infection, and increased seed yields by 25-100% at sites where blossom blight levels were moderate to severe. Application of chlorothalonil did not reduce flower infection, but yield increases at some sites were equal to those of benomyl. Similar results were obtained in a limited number of trials that included mancozeb. Iprodione and vinclozolin did not improve seed yields. The impact of fungicide application on foliar disease severity was generally small. We conclude that blossom blight occurs throughout the region, and that it causes significant yield loss in some years.

Alfalfa Yellows in Central Washington

Brian Lewis, Pioneer Hi-Bred Intl., Inc., Field Agronomist and Roland D. Meyer, Extension Soil Specialist, University of California-Davis

Alfalfa yellows was first studied in the 1950ís in Central Washington as a new problem. The study ended in the mid-sixties as researchers found no disease organism or plant nutrient deficiency associated with alfalfa yellows and no information was published from these early studies. Excessive subsoil moisture, due to seepage from water ditches and/or over-irrigation, was thought to cause alfalfa yellows. Alfalfa yellows symptoms are: Leaves are light green to yellow and distorted, especially terminal leaves. In severe cases, stunted plants may be only several inches tall. Alfalfa yellows symptoms may be confused with symptoms of other growth problems, such as bacterial wilt. The alfalfa yellows have been observed in all cuttings except the first. This studyís objective was to find a nutrient deficiency and/or soil moisture characteristic that may be associated with alfalfa yellows. Four locations were studied in Central Washington: three in 1996 and one in 1997. Two replications of a "non-affected" (green plants) site adjacent to an "affected" (yellow plants) site were studied at each location. Each site was approximately 20 feet X 20 feet. Soil and plant samples were taken from each site on the date when alfalfa growth was at least 12-15 inches tall. Sixty stems were collected from 30 plants. Soil cores were collected to a depth of 8 inches at all locations with the exception of one location where cores were 12 inches. Soil moisture cores were collected in one-foot increments to 3 feet to measure plant available water (1/3 and 15 bar) and actual moisture content. All samples were tested at the University of California DANR Laboratory at Davis, California. No significant differences were found between affected and non-affected sites for soil and plant nutrients. Soil moisture, expressed as "percent of field capacity", was significantly higher in the affected areas in the second and third foot depths. Soil moisture in the top foot was higher in the affected sites, and was not significantly different than the non-affected sites. This studyís results show that control of alfalfa yellows can be done by distributing water so that affected areas receive less-than-normal water and non-affected areas receive the same or more water. While water management is easy on fields with clear boundaries between affected and non-affected areas, most affected fields have these areas intermixed. For intermixed areas, check the amount and depth of soil moisture in non-affected areas before reducing applied water across a field. If water application is reduced to help control yellows in affected areas, non-affected areas must maintain equal production as before with less water. For this to happen, non-affected areas must have adequate soil moisture to 3 feet or more. If soil moisture is adequate to maintain production in non-affected areas, reducing water application rates by one-half or more may eliminate the alfalfa yellows caused from excessive subsoil moisture.

Alfalfa genetically transformed to produce resveratrol, a foreign antifungal compound

N.L. Paiva and J.D. Hipskind
Plant Biology Division, The Samuel Roberts Noble Foundation, P.O. Box 2180, Ardmore, OK 73402

Alfalfa naturally produces antifungal compounds (phytoalexins) such as medicarpin as part of its defense response to invading pathogenic fungi. However, many alfalfa pathogens have co-evolved mechanisms for avoiding the toxic effects of their hostís native phytoalexins. We have therefore introduced into alfalfa a gene encoding resveratrol synthase (RS) from peanut, which converts coumaroyl-CoA and malonyl-CoA to 3,5,4í-trihydroxystilbene, commonly known as resveratrol. Resveratrol is a phytoalexin found in a wide range of plants including peanut and grape, but is not found in alfalfa. The main goals of this work are to determine if this foreign metabolite accumulates to high levels or is rapidly degraded by alfalfa, to test whether or not accumulation of this foreign phytoalexin improves the resistance of alfalfa to any pathogens, and to check for any negative impact of resveratrol accumulation on plant health. Other labs have indicated that introduction of the RS gene from grape into transgenic tobacco conferred resistance to *Botrytis*, a weak tobacco pathogen (1). Strong expression of grape RS under the

CaMV 35S (cauliflower mosaic virus 35S ribosomal subunit) promoter resulted in high levels of resveratrol (via ELISA assay) in transgenic tobacco, but the plants had non-viable pollen and lost their pink flower color(2).

To date we have analyzed transgenic alfalfa plants expressing a resveratrol synthase cDNA clone from peanut, under the control of a very strong CaMV 35S promoter with a duplicated enhancer region. We do not observe the accumulation of any free resveratrol, but do detect very high levels of a resveratrol-glucose conjugate (resveratrol-3-O-beta-D-glucopyranoside, or "piceid"), using HPLC analysis. The concentration varies with the age of the tissues. For example, in higher accumulating lines, levels decrease from 150-170 ug/g.fr.wt. in younger leaves to 50 ug/g.fr.wt. in older leaves. On a molar basis, these levels equal or exceed the maximum levels observed for the natural alfalfa phytoalexins, and alfalfa pathogens were inhibited by resveratrol at these levels in agar-plate bioassays. Pathogen challenges of the transgenic plants are still in progress.

Unlike the reports for tobacco, high resveratrol-accumulating transgenic alfalfa plants show no visible differences compared to parent RegenSY and "empty vector" control plants, with respect to flower color, seed set following self-pollination, and nodulation by *Rhizobium meliloti*. A field test is currently underway to compare the levels of resveratrol observed in transgenics under greenhouse conditions with levels under field conditions. High levels of resveratrol and its glycosides are found naturally in wine and grapes, and may have beneficial effects on human health, including tumor-suppressing activities(3). We are pursuing animal tests to determine if a diet containing high resveratrol alfalfa is more beneficial than a diet supplemented with wild-type alfalfa.

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Identification and Utilization of Plant Protein Inhibitors for Insect Control in Alfalfa

T.C. Elden, A.C. Smigocki, S.E. Wilhite, G.R. Bauchan[1], and D.A. Samac[2]
[1]USDA-ARS, Beltsville, MD and [2]St. Paul, MN

Transgenic crop plants engineered for resistance to insect pests are now part of several pest management systems. Current insect resistance research deals primarily with the introduction of insecticidal proteins derived from soil bacteria or from plant species other than the one of interest. At present proteinase and alpha-amylase inhibitors and lectins are three classes of proteins in plants which we are investigating for their potential to function as chemical defensive factors against several major alfalfa pests. These insecticidal proteins are thought to be toxic in part by their ability to block digestive processes within the insects midgut, thus limiting nutritional proteins or essential amino-acids necessary for growth, development, or reproduction.

In vivo laboratory bioassays conducted in our laboratory have demonstratd that selected microbial and synthetic cysteine proteinase inhibitors significantly inhibited alfalfa weevil foliar feeding, pupation, adult emergence, and fecundity. Several plant lectins also were identified which significantly inhibited many of these same variables. Two cDNA clones of cysteine proteinase inhibitor genes from rice have been recloned into an *E. coli* expression vector for recombinant protein syntheses and their effects tested in vivo on alfalfa weevil growth and development and in vitro on alfalfa weevil midgut activity. These same genes have been reconstructed for expression in plant cells, cloned into transformation vectors carrying reporter and selectable marker genes and introduced into alfalfa explants via *Agrobacterium*-mediated gene transfer methods. Regenerated alfalfa transformants are currntly being vegetatively propagated and will be characterized for protein expression of the inhibitor genes by molecular techniques and in vivo laboratory insect bioassays.

Reports in the literature on other insect protein-inhibitor relationships suggest that insects utilize several classes of proteinases for digestion and that within a specific class there may be multiple forms controlled by different genes. In order to disrupt the digestive physiology of a specific insect it may be necessary to target multiple proteinases. Efforts are currently underway to identify multiple proteinase genes that may be involved in alfalfa weevil digestion so that these major proteinases can be targeted by inhibitors that are specific and potent. Towards this approach we have recently cloned a cysteine proteinase gene from the alfalfa weevil midgut and hope to identify additional cysteine proteinase genes. These genes will be used to express recombinant proteins for screening a library of novel cysteine proteinase inhibitors for selected variants with the highest binding affinity and insecticidal activity.

Biologic and economic assessment of insecticide use in alfalfa in the U.S.

A.A. Hower[1], J.K. Harper[2], and R.G. Harvey[3]
[1] Dept. of Entomol. and [2]Dept. of Ag. Econ. & Rural Soc., The Pennsylvania State University, University Park, PA. 16802, [3]Agron. Dept., University of Wisconsin, Madison, WI 53706

The primary management tactic for insect pests on alfalfa is the proper and timely application of insecticides. An assessment was conducted to determine the extent of their use on the nationís alfalfa crop (both hay and seed production), the biologic and economic significance of this use, and the effect of their withdrawal on alfalfa production. Data presented in the surveys covered the period from 1988 to 1992 and represented 99.9% of the 25.6 million hay acres. Data for the alfalfa seed crop represented seven western states growing 168,000 acres of seed alfalfa.

Twenty one insects or insect complexes were identified as requiring pesticide application on alfalfa hay. On average, 25.4% of the U.S. alfalfa, or 6.5 million acres, received annual insecticide applications. When considering multiple applications in a season, the treated acreage rose to 10.6 million acres. Insecticides were applied to 50.9% of the acreage using ground application equipment with the remaining 47.0% and 2.1% being applied by aircraft and chemigation, respectively. Organophosphates were the most common insecticide applied (59.4% of acreage). Carbamates ranked second (32.1%) and pyrethroids (5.3%) third. All treatments accounted for approximately 6.1 million lb. of insecticide per year. Carbofuran was the single most used insecticide followed by chlorpyrifos and dimethoate. Early harvest, host plant resistance, and natural biological control were the most common nonchemical management alternatives. Average efficacy of the 10 most widely used insecticides ranged from 3.4 to 4.6 based on a 5 point scale with 5 rated excellent.

Fourteen insects were identified as pest problems on alfalfa seed acres. The most important insects were Lygus spp. Propargite (a sulfonate) ranked first in both acres treated and volume of insecticide applied to the alfalfa seed crop. Host plant resistance was the most common noninsecticide management practice.

The loss of alfalfa insecticides could have substantial negative impacts on farm-level profitability. Overall, the negative economic impacts would be most keenly felt in the western U.S. because of its large hay acreage and seed production. The North Central U.S. would also experience large negative economic impacts, mainly because this region has the greatest alfalfa hay acreage. Losses of at least $1 million would result from the banning of 12 insecticides. The greatest negative economic impact would result from the loss of carbofuran ($68.5 million), followed by dimethoate ($41.4 million) and methomyl ($33.7 million). As a class, the banning of organophosphates would result in a loss of $103.4 million. If all chemical insecticides were banned in alfalfa production, an annual loss of $331.9 million would result (if cultural practices cost $30/A). In this case, loss of insecticides would have a negative impact of $44/acre for hay production and $368/acre for seed production. A ban on an insecticide would reduce the options available to farmers, forcing the substitution of insecticides which are usually either more expensive, less effective, more difficult to use, or less adaptable to changing production conditions. The profitability of alfalfa production would be seriously affected by the banning of individual pesticides and possibly be eliminated as a crop alternative if all chemical pesticide options were to be banned.

Subcellular localization of two enzymes in medicarpin biosynthesis in alfalfa

Melina López-Meyer and Nancy L. Paiva.
The Samuel Roberts Noble Foundation. P.O.Box 2180,Ardmore, OK, 73402

Alfalfa plants respond to pathogen attack by producing phytoalexins. In this plant, the major phytoalexin is medicarpin. This compound is induced in leaves that have been challenged with fungal pathogens, but not in healthy leaves. Also, medicarpin can be conjugated to malonyl and glucosyl residues to form MGM, which accumulates in roots and nodules. These observations indicate that medicarpin might be playing an important role in the defense system of the plant against fungal attack. Medicarpin is synthesized via the isoflavonoid branch of phenylpropanoid metabolism. Isoflavone reductase (IFR) and vestitone reductase (VR) are two enzymes involved in the last part of the pathway (Guo et al.,1994; Oommen et al.,1994. Very little is known about the subcellular location of isoflavonoid-specific enzymes. Since modification of phytoalexin composition in alfalfa represents a potential method for increasing pathogen resistance, knowledge of the compartmentation of the enzymes in the pathway may have a major impact on such modification strategies. Thus, the aim of this work was to determine the subcellular location of IFR and VR in *Phoma medicaginis*-infected alfalfa plants. For this purpose, polyclonal antibodies raised against IFR and VR proteins were used in immunocytochemical studies. As anti-VR serum showed a single band in western analysis of infected leaf protein extracts, this serum was used to immunolocalize VR protein by transmission electron microscopy. It was determined that VR is a cytoplasmic enzyme. Western blot analysis of infected alfalfa leaf extracts challenged with anti-IFR serum showed the presence of two major and two minor bands. As this antiserum was not suitable for use in immunolocalization studies in alfalfa, transgenic tobacco plants transformed with the alfalfa IFR cDNA under the regulation of the CaMV35S promoter were used for these studies. Anti-IFR serum showed a single band in western analysis of 35S::IFR tobacco leaf extracts. Electron microscopy analysis of IFR-expressing tobacco leaves showed that IFR is also a cytoplasmic enzyme. Additional analysis of infected alfalfa leaf sections challenged with anti-IFR serum revealed that all the signal detected was localized in the cytoplasm. This confirmed the cytoplasmic localization of IFR in alfalfa, and showed that the other proteins that were recognized by anti-IFR serum were also cytoplasmic. The data presented in this work indicate that, at least up to the penultimate biosynthetic step catalyzed by VR, the biosynthesis takes place in the cytoplasm. The location of the latest step in the medicarpin pathway and the conjugation of medicarpin to MGM remain to be elucidated.

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U.S. *Medicago* Germplasm Collection: Celebrating a Century of Plant Collecting, Introduction and Conservation

Stephanie L. Greene
USDA, National Plant Germplasm System, Washington State University, Prosser, WA 99350.

The year 1998 marks the centennial for many USDA germplasm collections in the United States. In 1898, the USDA Division of Botany, Section of Seed and Plant Introduction began assigning Plant Introduction (PI) numbers to seed and plants brought into the United States. The first alfalfa PIs were collected in Russia and Central Asia in 1897 by the plant explorer, N.E. Hansen, a horticulturalist at the South Dakota Agricultural Experiment Station. The source of much of the *M. sativa* ssp. *falcata* and *M. sativa* nothossp. *varia* germplasm used in the United States can be traced back to these specific seed lots. In fact, most of the nine germplasm sources that contributed to the development of alfalfa in the United States, can be traced back to specific seed lots brought into the country through the Plant Introduction program. Today, the *Medicago* germplasm collection contains more than 7,100 accessions, representing 72 of the 85 species in the genus. Reflecting a historic mandate of introduction, 90 % of the collection represents the cultivated gene pool of either alfalfa or the annual species. The U.S. collection plays a limited role in the *ex situ* conservation of the non-cultivated primary and secondary genepool. The table below compares the number of accessions that represent the primary and secondary alfalfa (*Medicago sativa ssp. sativa*) gene pool.

Category	ssp. *sativa*	ssp. *falcata*	nothos sp. *varia*	nothos sp. *polychroa*	*M. prostrata*	ssp. *glomerata*	nothossp. *tunetana*	ssp. *caerulea*	*M. papillosa*
Breeding	**358**	**11**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Cultivar	**787**	**21**	**106**	**0**	**0**	**0**	**0**	**0**	**0**
Cultivated	**288**	**50**	**14**	**0**	**0**	**2**	**1**	**16**	**0**
Landrace	**614**	**20**	**63**	**0**	**0**	**0**	**0**	**0**	**0**
Uncertain	**386**	**18**	**17**	**0**	**0**	**0**	**0**	**16**	**0**

Wild	**366**	**276**	**76**	**0**	**13**	**5**	**5**	**45**	**9**
	2799	**396**	**276**	**0**	**13**	**7**	**6**	**77**	**9**

(GRIN, June 1998. Taxonomy based on Wiersma et al. 1990)

Annual species in the tertiary gene pool are well represented, largely due to the inclusion of the K.A. Lesin's collection in the 1980's. However, only 13 perennial species are represented in the tertiary gene pool. An extensive revision of the NPGS *Medicago* database has been recently completed and is available to the public at the GRIN website (http://www.ars grin.gov/npgs). Seed requests can also be made at the website. The extensive upgrade of the passport information will provide the collection curator and CGC members the opportunity to examine the contents of the collection at a level of detail not previously possible. As the century draws to a close, it seems appropriate to carry out such an examination to ensure the U.S. *Medicago* germplasm collection effectively serves the dual role of conserving and encouraging the use of diverse *Medicago* genetic resources in the 21st. Century.

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Distribution of size polymorphisms in hypervariable chloroplast DNA regions in alfalfa

D. Z. Skinner and M. M. Hetzel
USDA-ARS and Agronomy Department, Kansas State University, Manhattan, KS 66506-5501

Two hypervariable regions have been identified in the alfalfa (*Medicago sativa* L) chloroplast genome. These regions initially were identified through restriction enzyme analysis and therefore are identified as the *Hin*dIII site and the *Hae*II site, the enzymes first used to discover their presence. The *Hin*dIII region was discovered as a hypervariable region within the *Medicago sativa* species (Schabel, 1996). The *Hae*II region was originally identified as hypervariable between *Medicago* species (Johnson and Palmer, 1989). After their discovery, through cloning and sequencing, PCR primers flanking the hypervariable restriction sites were designed, and now are used routinely to assay the size of fragment(s) represented in individual plants.

Variability at the *Hin*DIII site is assayed by the amplification of a fragment about 800bp in length, while the *Hae*II site amplification yields a fragment of about 1100bp. Rounded to the nearest 50 bp, the *Hin*dIII fragment ranged from 200 to 1150bp, and the *Hae*II fragment ranged from 550 to 3950bp, with the frequencies shown in Table 1.

Comparisons of the polymorphic fragment distributions between PI lines from the *Medicago sativa* core collection yielded estimates of genetic distances between the PI lines. Principal component analysis of the distance data was used to develop a representation of the realtionships of the of PI lines and countries of origin. In general, the *Hin*dIII and *Hae*II regions yielded similar results. Specific size polymorphisms tended to be associated with geographic regions from which the PI's were collected. However, the distribution of polymorphic fragments in PI's collected from outside of the region of alfalfa origin, such as the US and Canada, tended to be much more heterogeneous. As a result, these PI lines often appeared to be more-or-less equally related to PI's from several geographic regions.

Table 1. Size and frequency distributions of DNA fragments generated by PCR amplification of two hypervariable regions in the *Medicago sativa* chloroplast genome.

***Hae* II region**			***Hin*D III region**		
size(bp)	**count**	**frequency(%)**	**size (bp)**	**count**	**frequency(%)**
550	**1**	**0**	**200**	**2**	**0.1**
650	**2**	**0.1**	**250**	**4**	**0.1**
750	**3**	**0.1**	**300**	**10**	**0.4**

800	2	0.1	350	176	6.3
850	9	0.2	400	181	6.5
900	7	0.2	450	202	7.2
950	2	0.1	500	173	6.2
1000	12	0.3	550	354	12.7
1050	63	1.7	600	194	6.9
1100	50	1.4	650	131	4.7
1150	259	7.1	700	178	6.4
1200	716	19.6	750	257	9.2
1250	936	25.6	800	252	9
1300	857	23.4	850	228	8.2
1350	495	13.5	900	215	7.7
1400	143	3.9	950	143	5.1
1450	44	1.2	1000	71	2.5
1500	19	0.5	1050	22	0.8
1550	16	0.4	1100	1	0
1600	9	0.2	1150	2	0.1
1650	1	0			
1700	1	0			
1750	1	0			
2100	1	0			
2200	1	0			
3250	1	0			
3650	1	0			
3750	1	0			
3800	2	0.1			
3850	2	0.1			
3900	2	0.1			

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Isolation and Characterization of a ßAmylase Gene from Alfalfa

J.A. Gana, S.M. Cunningham, and J.J. Volenec
Dept. of Agronomy, Purdue University, West Lafayette, IN 47907-1150

Alfalfa (*Medicago sativa* L.) roots contain large quantities of ß -amylase, but the role of this enzyme *in planta* is poorly understood. We have speculated that ß-amylase may be a storage protein providing regrowing shoots with N. This suggestion is based on several line of reasoning: 1) preferential accumulation of ß -amylase in storage taproots to between 5 and 8% of root soluble protein; 2) its disappearance from roots when shoot growth resumes in spring and after defoliation; and 3) no association between increases in ß -amylase activity and enhanced starch utilization. We studied the role of ß -amylase by isolating a ß -amylase cDNA and examining factors that affect expression of its gene. The ß -amylase cDNA encoded a 55.95 kD polypeptide with deduced amino acid sequence showing high similarity to other ß -amylases, especially those from soybean seeds. Starch concentrations, ß -amylase activity, and ß -amylase mRNA levels were measured in roots of alfalfa after defoliation and during cold-acclimation. Starch levels, ß -amylase activity, and ß -amylase transcripts were reduced significantly in roots of defoliated plants. The ß -amylase transcript was high in roots of undefoliated plants, but disappeared from roots within 2 d after defoliation and remained very low until 12 d after defoliation. ß -amylase transcript levels increased in roots of germplasms between September and October, then declined markedly in November and December after shoots were killed by frost (Fig. 1).

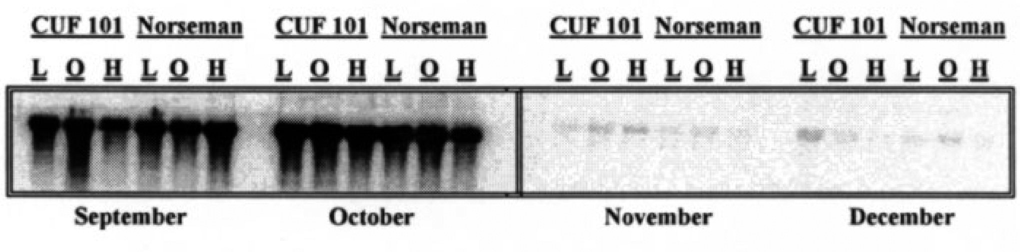

Figure 1. Cold acclimation effects on ß -amylase transcript levels in six alfalfa germplasms. The cultivars CUF 101 (O) and Norseman (O) were selected for three cycles for more (L) or less (H) fall dormancy. Roots of field-grown plants were sampled in the months indicated.

Expression of ß -amylase in alfalfa is tissue-specific, with very high expression in roots, moderate expression in stems, and very low expression in leaves. Alfalfa roots contain high ß -amylase transcript levels when compared to roots of sweetclover and red clover, with transcript levels below our detection limit in birdsfoot trefoil. Southern analysis indicates that ß -amylase is present in alfalfa as a multi-gene family. Our evidence indicates that ß -amylase is not involved in starch hydrolysis in alfalfa roots but may function as a storage protein.

Seed Productivity of Native and Foreign Alfalfa Varieties Under Conditions of Radial Seeding

M. Voloshin, V. Valter and T. Voloshina
Alfalfa Breeding Department, Lukyanenko Research Institute of Agriculture, Krasnodar, Russia 350012

Numerous alfalfa seed growing researches in the south of Russia on relationship of the alfalfa stands structure with the seed productivity show that plant morphology and seed yield change under the influence of plant density.

Our experiments were carried out to investigate the structure of alfalfa stands in ordinary row (15 cm), wide-row (45 and 70 cm) and radial (1-140cm) sowings. Results showed that plant density mostly decreased in the first year of growth, especially in ordinary row sowings - up to 38%.

Significant difference between plant age and methods of sowing was established on the base of seed yield structure. Wide-row sowings of all alfalfa varieties possessed an advantage over ordinary row sowings. They considerably surpass plants in ordinary sowings in number of inflorescences, flowers per one shoot; seed formation was higher by 20-26%. The alfalfa seed yield in the second year was in ordinary row sowings - 340 kg/ha, in wide-row sowings - 610 kg/ha. In the forth year it was 200 kg/ha and 320 kg/ha, correspondingly.

Radial sowings were more convenient for observation and evaluations. For such sowings we outlined a circle with 5 m radius which was marked to the maximum width of rows. In our experiment it was 140 cm. The seeder CP1 moved from the center of the circle towards the circumference so we obtained 22 plots with row width from 1 to 140 cm, 120 seeds/linear metre. The study of seed productivity and fresh yield was carried out in every five meters. The first five metres conformed to row width 1-28 cm, the second five metres to 29-56 cm, the third to 57-84 cm, the forth to 85-112 cm, the fifth to 113-140 cm.

Table. Alfalfa Seed Yield in Radial Sowings in Anapo-Taman Region. Average for 3 Years

Cultivar, number	Seed yield, g/m^2, with row width				
	1-28 cm	29-56 cm	57-84 cm	85-112 cm	113-140 cm
Bagira	33	47	59	63	56
171h283	36	49	63	58	47
Globus	25	36	57	43	44
Polar	20	35	49	52	46
Drymmor	24	30	51	55	47

MEB 8306	**24**	**40**	**53**	**47**	**39**
Krona	**28**	**42**	**68**	**82**	**73**
$LSD_{0,05}$	**3**	**3**	**4**	**3**	**4**

The data of alfalfa fresh yields obtained showed the advantage of alfalfa sowings with the row width 1-28 cm and 29-56 cm. Sowings with wider rows were inferior to the above sowings in dry years by 5-47%, in wet years - up to 65%. The results of alfalfa seed productivity showed that the best sowings were in the central zone of Krasnodar region with row width 29-84 cm, in Anapo-Taman zone with row width 57-112 cm.

The Alfalfa Germplasm Resources, Present Situation of Planting Consensus and their Outlook of Breeding and Selection on Loess Plateau of China

Shi Yongjie and Wu Renrun
Lanzhou Institute of Animal and Veterinary Sciences, Chinese Academy of Agricultural Sciences, Lanzhou, Gansu. People's Republic of China

Alfalfa has been cultivated in China ever since its introduction from Persia (now Iran) about 200 years ago. In China alfalfa is primarily planted in the Loess Plateau area which includes 7 provinces including Gansu, Qinghai, Ningxia, Shaanxi, Shanxi, Henan and Neimenggu Provinces. Altogether the Loess Plateau includes 431,580 km2 . The total area of alfalfa production in China is 1.35 million ha, whereas, the Loess Plateau area contains 860,000 ha of alfalfa according to the P.R.C. Ministry of Agriculture's most recent information. The total area of production of alfalfa in China has increased over the years, 508,000 ha in the 1950's, 643,000 ha in the 1960's, 805,000 ha in the 1970's, 1.33 million ha in the 1980's and 1.35 million ha in the 1990's. The alfalfa production area in the Loess Plateau area has increased markedly from the 1950's where there were only 271,000 ha to today there are 861,000 ha. China has a number of *Medicago* species distributed throughout the Loess Plateau area which grow in the wild, were introduced or are cultivated. The perennial species include: *M. alaschanica, M. arborea, M. archiducis-nicolai, M. caerulea, M. falcata, M. varia, M. sativa, M. vassilczenkoi*, and *M. ruthenica*. The annual species include: *M. arabica, M. littoralis, M. lupulina, M. minima, M. polymorpha, M. schischkini, M. tornata*, and *M. truncatula*. Prior to the requirement at all alfalfa varieties need to be licenced, China had 177 land races which were distributed among 5 provinces (Shanxi, Shaaxi, Ningxia, Gansu, and Quinghai) and the Loess Plateau with the Loess Plateau area containing the largest number of 61. Modern Chinese alfalfa varieties have been licenced through the Chinese Herbage Cultivar Registration Board. these include 5 variegated alfalfa varieties (Caoyuan No. 1, Caoyuan No.2, Gannong No. 1, Rambler, and Xinmu No.1) and 18 purple flowered varieties (Aohan, Beijiang, Cangzhou, Gongnong No.1 Gongnong No.2, Guanzhong, Hexi, Huaiyin, Jinnan, Longdong, Longzhong, Neimeng Zhungeer, Shaanbei, Tianshui, Tumu No. 2, Xinjiang Daye, Yuxian, and Zhaodong). The province of Gansu has been and will continue to be an important geographic center for the scientific research of alfalfa. Future research must follow 3 patterns: 1) materialization, 2) systematization and 3) standardization in accordance with the theory of Agricultural engineering in a rigorous scientific approach, thus we can pass through the hall into the inner chamber and easily attain our final goal. There are two areas we are focusing on: one is fall dormancy/winter hardening and the second is resistance to downy mildew. In the area of fall dormancy and winter hardiness, Lu Xinshi has worked with Larry Teuber resulting in their report on the topic of Allozyme Characterization of the National Alfalfa Cultivars from the People's Republic of China. In this study they analyzed 23 officially approved National Chinese cultivars and 9 US cultivars (Lu Xinshi, et al 1992). At present we are conducting the same type of allozyme study in Lanzhou. In the area of downy mildew resistance we are also continuing to fight against downy mildew. We estimated that downy mildew has reduced the yield of alfalfa by 30%. Please refer to Ma and Hou (1994) report on "The study on selection and breeding of alfalfa new clutivars resistant to downy mildew in China".

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Comparison of Variation at Molecular Markers And Quantitative Traits In Alfalfa.

Sergey Ivashuta, Kazuhiro Uchiyama, Mitsuru Gau and Sachiko Isobe

Hokkaido National Agricultural Experimental Station, Toyohira-ku, Sapporo 062-8555, Japan

RAPD analysis combined with AMOVA (Analysis of Molecular Variance) were used for assessment of genetic variation within and between two alfalfa populations of contrasting freezing tolerance and fall dormancy (var.Moapa 67 and var.Rambler). The analysis revealed high level of variation within populations and relatively low level of differentiation between populations. Of the total genetic diversity about 80% was attributed to the individual differences within a populations and less than 20% - to the between-populations variance components. Meanwhile, there were notable differentiation of these populations in the freezing tolerance and fall dormancy as revealed by nested ANOVA. A comparison showed that there is little congruence between patterns of variation at molecular marker loci and in quantitative traits under study.

Generally, the similar level of RAPD polymorphism was revealed between any two alfalfa plants drawn from same population (similar freezing tolerance and fall dormancy) or from different populations (differing significantly in freezing tolerance and fall dormancy). We did not find population-specific RAPD markers and only several bands were found that had significant differences in frequency between two populations. Another class of molecular markers, namely AFLP, also showed high level of genomic DNA polymorphism between any two alfalfa plants. On the contrary, when ds cDNA was used as a template for AFLP reaction level of "polymorphism" was significantly lower. cDNA was synthesized from mRNA isolated from genotypes of contrasting freezing tolerance grown under normal and stress conditions (cold treatment). The fragment was counted as "polymorphic" if it present in at least one mRNA samples (non-hardened or hardened plants) of one genotype and absented in both mRNA samples of another genotype. In our preliminary experiment, on average less than 5% of the total number of bands were different between plants from different populations. This is several times lower as compared with level of polymorphism that was revealed for genomic DNA. In addition, it is evident that only some of the "polymorphic" bands displayed in the cDNA-AFLP experiment are truly polymorphic on genomic level (rather than differentially expressed genes) as could be proven by Southern hybridization.

We suppose that DNA polymorphism of the expressed sequences detected with cDNA-based markers (used as a probe or after converting to DNA-based PCR markers) can more clear reflect the pattern of variation in quantitative traits in outcrossing species. These markers would be also useful for genetic map construction and MAS.

RAPD Analysis in Alfalfa Cultivars of Contrasting Freezing Tolerance

K. Uchiyama, S. I. Ivashuta, M. Gau and S. Isobe
Hokkaido Natl. Agric. Exp. Sta., Hitsujigaoka, Toyohira-ku, Sapporo 062-8555, Japan

We are trying to find DNA markers associated with freezing tolerance and/or fall dormancy of alfalfa using F_2 and BC_1F_1 populations between cv. Rambler (freezing tolerant) and cv. Moapa 69 (freezing sensitive). To reduce labor and cost in analyzing the segregating populations, in which we have to deal with a lot of plants, we preliminarily screened the RAPD markers (Operon) using parental clones and cultivars, and other cultivars of contrasting freezing tolerance. We are trying to find the DNA markers of greater or lower frequency in freezing tolerant cultivars or sensitive ones or specific to them. We have done several approaches for that purpose.

1) We used 2 genotypes each from Moapa 69 and Rambler including parental clones for producing F_2 and BC_1F_1 populations. We screened RAPD markers common in one cultivar and absent in the other one. 251 out of 316 primers showed polymorphism possible to analyze and 804 polymorphic bands were observed. There were 131 bands (16.3%) specific to Moapa 69 and 117 bands (14.6%) specific to Rambler.

2) Using 36 cultivars (10 plants bulk) of contrasting freezing tolerance, we screened 20 primers for the bands of greater frequency in cold tolerant cultivars or sensitive ones. We observed 220 total bands and 169 polymorphic ones (76.8%). We found 2 markers each of greater frequency in cold tolerant cultivars or in sensitive ones.

3) Using 10 plants each from 9 cultivars (each 3 cultivars from cold sensitive, moderately tolerant and tolerant ones), we screened 5 primers for the bands of higher frequency or lower frequency in cold sensitive or tolerant cultivars. We observed

77 total bands and 5 bands were adapted to our purpose.

Using F_2 progeny between Rambler and Moapa 69 and BC_1F_1 progeny backcrossed to Rambler, now we are analyzing the segregation of freezing tolerance and DNA markers. We will check whether our pre-screening strategies are effective or not in the segregating populations.

Performance of diallel crosses of alfalfa with different levels of genetic diversity and derived from partly inbred parents.I. Seed setting and pod fertility.

P. Rotili*, C. Scotti*, D. Kertikova and G. Gnocchi*.**
*** Istituto Sperimentale per le Colture Foraggere, Lodi, Italy.**
**** Forage Crops Institute, Pleven, Bulgaria.**

CGA, SCA, GRE and SRE are estimated in 5 diallel crosses derived from 8 parental S2 families. In the first diallel cross (2.21 and 54 mean values for pod fertility and seed setting respectively) the 8 parental families have the same genetic origin (cv Lodi); in the second diallel cross (2.95 and 63 mean values) they have the 50% of the genetic diversity (cvs Lodi and La Rocca). The last 3 diallel crosses have 100% of the genetic diversity, and precisely: the third diallel cross (3.03 and 63 mean values) derived from the cvs La Rocca, Sprinter, Selene, Shell, Birouk, Robot, Romea, Siwa; the fourth diallel cross (2.81 and 64 mean values) derived from the cvs Oro, Prosementi,Odessa, Supreme, Estival, Natsuwa, Amiral, Tunetana; the fifth diallel cross (2.88 and 57 mean values) derived from the cvs Cimarron, Vitroculture, Gilboa, Delta, Boreal, Diablo verde, Kara, Lodi. This study is the first phase of a program concerning the construction of semi-hybrid varieties. Seed-setting (percentage of pod per tripped flowers) and pod fertility of the parental plants are measured. In the diallel cross progenies the following characters are studied: height, number of stems, flowering degree, dry weight.

On this paper only the seed-setting and pod fertility results are reported. The crosses were made by hand in the greenhouse without emasculation. The variance analysis was made by Method III Model I of Griffing. The results for seed-setting and for pod fertility show:
1) the importance of parental genetic diversity. Notice that a diversity of 50% gives the same results of a diversity of 100%. 2) General Combining Ability (GCA) was much larger than Specific Combining Ability (SCA) in both characters. Some cases of SCA could be utilized for the choice of single crosses for the construction of double hybrids. At the same time we can observe some negative cases of SCA. 3) General Reciprocal Effects (GRE) were much larger than Specific Reciprocal Effects (SRE). This means that in the selection of single crosses the direction of the crossing is very important (Rotili and Zannone, 1977). 4) The positive correlation between pod fertility and seed-setting will allow us to utilize these two parameters in the selection for seed production. These results suggest that the polycross method in the construction of synthetic varieties could give negative results or results of little efficiency for the seed production and for the forage production. This method, even without the selective effect of pollinators, does not permit to know the reproductive possibilities of an individual plant of the polycross group. The real situation is very far from panmictic conditions because it is very frequent to have a high variability among parental plants for the quantity and quality of pollen that influence the pollen competition. Therefore even in the construction of synthetic varieties a better solution is to utilize the diallel cross method realized by hand without emasculation.

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Quantitative Ovule Sterility in *Medicago sativa*

Daniele Rosellini[1], Franco Lorenzetti and Edwin T. Bingham[2]
[1]Istituto di Miglioramento Genetico Vegetale, Università degli Studi di Perugia, Borgo XX giugno 74, 06100 Perugia, Italy. E-mail roselli@krenet.it
[2]Department of Agronomy, University of Wisconsin-Madison, 1575 Linden Dr., Madison, WI, USA 53706

Seed/ovule ratio per unit area in the field is about 8% in alfalfa (1). Among the possible mechanisms of low seed set, embryo abortion has been demonstrated. However, it is not known whether pre-fertilization ovule sterility plays a role. Only a single factor female sterility trait has been described to date (2).

We investigated the inheritance of an ovule sterility trait associated with callose (b1,3 and b1,4 glucan) deposition in mature ovules and studied its occurence in six alfalfa populations. All the plants used were grown in pots in a greenhouse in Madison, Wisconsin, USA, from November 1996 to July 1997 under continuous light. Florets were sampled at flowering, fixed in 70% ethanol:acetic acid 3:1 for at least 24 hours. The pistils were then dissected, cleared in 8N NaOH for 5 hours, stained overnight in 0.1% aniline blue in 0.1M K3PO4, mounted in the staining solution and gently squashed under a coverslip. Fluorescence microscopy using UV light was then used for evidencing callose.

Ovule sterility was found to be associated with callose deposition in B17, a plant with low fertility from the alfalfa cv Blazer XL. The site of callose deposition, which began during embryo sac development and affected 81% of the ovules in mature florets, at random positions in the ovary, appeared to be the embryo sac wall or the integumentary tapetum. B17 was crossed with P13, a Peruvian plant with 5% callosized ovules, to generate reciprocal F1 populations, and an F1 plant (91% callosized ovules) was used to obtain the backcross populations. B17 was also crossed to unrelated, highly fertile plants. S1 progenies from B17 and P13 were also studied.

The fertile ovules of B17 transmitted the ovule sterility trait to the progenies, thereby demonstrating a sporophytic genetic control. All the progeny populations displayed continuous variation for the percentage of sterile ovules, indicating that more than one gene is involved. Reduced transmission of the sterility trait through the pollen is hypothesized to explain the difference between reciprocal crosses. Six progeny plants showing 100% callosized ovules proved to be female sterile. Narrow-sense heritability estimated by offspring-midparent regression was 0.85.

Together, our results demonstrate a quantitative sporophytic additive inheritance of ovule sterility.

The percentage of sterile ovules per plant was determined on 22 to 25 plants from each of four North American and an Italian cultivar, and a tetraploid *M. falcata* population. On average, 4 to 24% sterile ovules per plant were found in these populations, and plants with up to 75% were observed. Ovule sterility was negatively correlated with the number of seeds per pod with both hand-crossing and selfing. This suggests that seed yield of cultivars could be adversely affected by ovule sterility, and that assessing this trait in base genotypes for cultivar development could help to obtain cultivars with better seed yield.

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ESTIMATION OF INBREEDING EFFECTS IN AN ALFALFA BREEDING PROGRAM: VIGOUR AND VARIATION IN HETEROZYGOSITY LEVEL BY RFLP MARKERS

Scotti C.[1] ,Salvi S.[1] ,Pupilli F.[2] and S.Arcioni[2]
[1] Istituto Sperimentale per le Colture Foraggere, V.le Piacenza 29, 26900 Lodi, Italy
[2] Istituto per il Miglioramento Genetico delle Piante Foraggere del CNR, Via Madonna Alta 130,06128 Perugia, Italy

The alfalfa breeding method set up at Lodi Institute provides for a phase of reduction of heterozygosity level through recurrent selfing and selection within selfed families. Some critical aspects of the selfing phase refer to: a)the knowledge of the starting point, that is the estimation of inbreeding coefficient of the parental non inbred plants; b) the effect of selection for vigour within selfed families. In fact, the additive value of genes and linkats implied in vigour and the average heterozygosity level both take part in phenotypic vigour expression (dry matter production in time). To estimate the variation of heterozygosity level during successive selfing generations as well as within each selfing generation, RFLP markers have been used.

Plant material is constituted by 6 S_1 families (90 plants in total, randomly chosen within each family), 9 S_2 families (166 plants in total, in two groups of opposite vigour within each progeny) derived from selection of plants in the previous generation, and 5 S_3 families (39 plants in total) originated from selected S_2 plants. Single plants were grown in 5 cm diameter PVC tubes (density of about 500 plants m^{-2}), with not limiting water supply and cut every 30 days. Dry matter production of S_1 and S_2 families was compared with polycross families (S_0 x S_0) obtained from the corresponding parental plants; S_3 families were compared with the corresponding S_2 x S_2 families. As for the estimation of heterozygosity level, plant DNA digested with Hind III was hybridised using 11 low copy number probes coming from a partial gene library of *Medicago sativa* (77 different fragments generated in total). The total number of fragments over the whole loci on individual plant basis was compared at every selfing level with the corresponding mother plants (MP) in the previous generations.

The total number of bands decreased from 41.17 (average of S_0 MP) to 37.22 (-9.55%) in S_1 and 34.38 (-15.68%) in the whole S_2 families. Considering the progenies having a S_3 generation, the decrease observed ranged from 42.75 (S_0 MP) to 38.55 (-9.82%) in S_1, 35.69 (-16.55%) in S_2 and 34.78 (-19.46%) in S_3. These results seem to be consistent with the reduction of 2nd and 3rd order allelic interactions in the first selfing generation and the increased frequency of diallelic genotypic structures in the subsequent ones. Inside each S_2 group of opposite vigour, interplant variation for the total number of fragments is sufficient to allow the choice of high performing individuals with different estimated heterozygosity level.

No consistent correlations between estimated heterozygosity level and vigour were found. In fact, the two groups of opposite vigour within each S_2 family did not differ significantly for the estimated heterozygosity level, indicating that different genotypic structures were randomly distributed in the two groups.

Genetic variation for male and female fertilities in alfalfa

Christian Huyghe, Rafik Leneguer, Pierre-Henri Auriel, Christel Bodin, Christian Ecalle, Bernadette Julier
INRA, Station díAmélioration des Plantes Fourragères,
F-86600 Lusignan, FRANCE

The seed production in alfalfa is influenced by the fertility of both male and female gametophytes. A lot of efforts have been devoted to the analysis of the pollen fertility but little to the ovules.

To assess the female fertility, a rapid clearing technique based on a fixation with FPA50, rinsing with ethanol and clearing with methylsalicylate was developed. It makes it possible to observe the embryo sac through the ovary structure without any dissection, using a phase contrast microscope. The pollen fertility was measured with Alexander staining.

The position of the flower into the inflorescence did not modify neither pollen nor ovule fertility. The position of the inflorescence on the stems only influenced the female fertility of a single genotype. The position of the ovule within the ovary did not influence the female fertility (figure 1).

The genetic variation available for both characters was analysed on 10 populations, with 9 individual plants per population. The observations were repeated over two years. This analysis confirmed the existence of a wide range of genetic variation for the pollen fertility (from 20 to 96% with an average of 69%). A wide range was also observed for the female fertility (from 36 to 98% with an average of 82%). For both fertilities, the within-population variance was either equal or larger than the between-population variance (Table 1). There was no strict relationship between the male and female fertilities (r=0.40, 88 df).

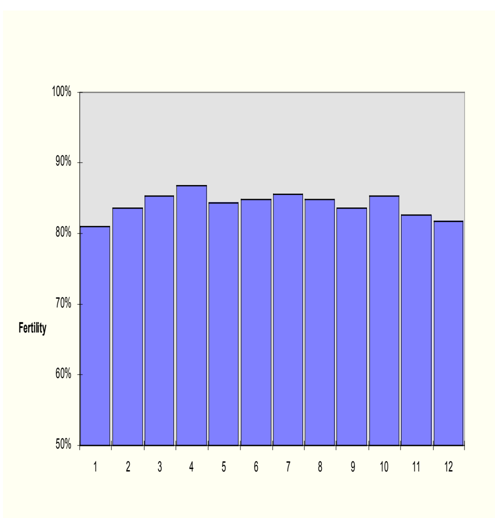
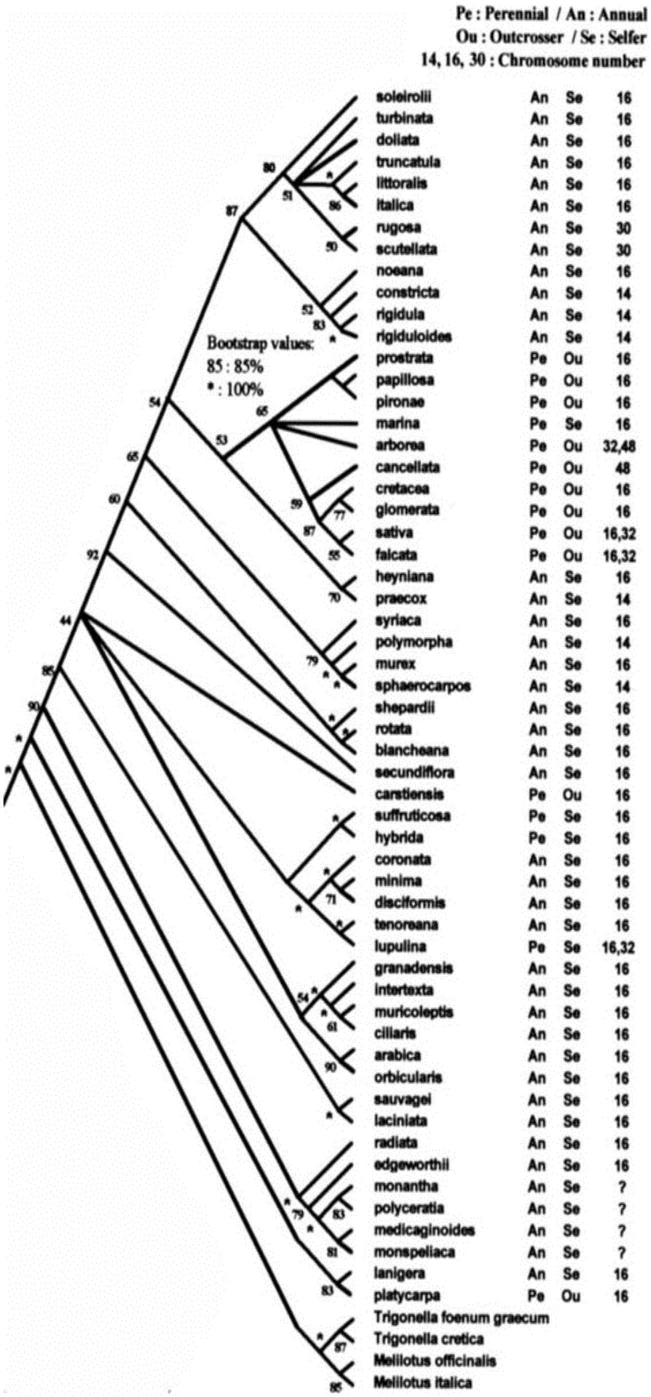


Table 1 : Partitioning of the genetic variance for male and female fertilities within and between 10 populations of alfalfa

	Male	Female
Between-pop	0.007	0.008
Within-pop	0.022	0.010
Error	0.004	0.011

MOLECULAR PHYLOGENY OF THE GENUS *Medicago*, TAXONOMIC AND EVOLUTIONARY IMPLICATIONS.

Gilles Bena [#$] , Jean-Marie Prosperi [#], Isabelle Olivieri[#$],Bernard Lejeune [#]Laboratoire de Ressoures Genetiques et d'Amelioration des Luzernes Mediterraneennes, INRA Montpellier, Mauguio, France Institut de Biotechnologie des Plantes, Universite Paris Xl Orsay, Flance [$] lnstitut des Sciences de l'Evolution, Universite Montpellier II France bena@isem.univ-montp2.fr, prosperi@ensam.inra.fr



Strict Consensus of the maximally parsimonious trees

Outgroups : Trigonella (2sp.), Melilotus (2sp.), Trifolium (2sp.), Astragalus (3sp.) and Vigna (1sp.)

We present a molecular phylogeny including most species of the genus *Medicago* L. This phylogeny is based on the sequences of ITSl and ITS2 (nuclear ribosomal internal transcribed spacers) and ETS (external transcribed spacer) [(3)], and includes 56 species. Based on the strict consensus of the 48 most parsimonious trees, an evolution of the genus is proposed, and taxonomic implications are discussed. Major incongruences were found between phylogenetic relationships and morphological classification of the genus [(4)]. Most of the sub-genera revealed to be paraphyletic. However, the reconstruction confirms the assignment of the medicagoid species from the genus Trigonella to Medicago [(1,2)]. Life history [(5)], morphological and cytological traits were mapped onto the phylogeny and their evolution studied.

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MEDICAGO TRUNCATULA : A model-plant

J.M. Prosperi∇ , I. Bonnin∇ ,M.Ghérardi# , E. Jenczewski∇ and T. Huguet #
 # LBMRPM, CNRS-INRA, BP27, 31326 Castanet-Tolosan Cedex, France.
∇ SGAP, INRA, Domaine de Melgueil, 34130 Mauguio, France.

Medicago truncatula is an annual forage, diploid, selfing plant of low DNA content and with which transgenic plants can be routinely obtained. This Mediterranean forage legume is used as a model-plant instead of alfalfa (*Medicago sativa*) to combine analyses of the genetic diversity and studies of molecular biology. *M. truncatula* is nodulated by well-known *Rhizobium meliloti* strains and is of good agronomical value for Mediterranean regions where they are used to improve the soil structure, increase soil nitrogen and as a source of winter forage.

Genetic resources and diversity of natural populations. Since 1985, more than 300 natural populations have been collected from 9 countries (North-Africa and Europe) and using agronomic and electrophoresis tools (isozymes), a core-collection of 130 populations was built[(1)]. A high level of polymorphism was detected both between and within natural populations for both neutral markers (RAPD) and morphological traits[(1,2,3,4)]. Concurrently, a selection of medics for introduction in Mediterranean marginal lands started and one cold tolerant variety was selected[(6)].

Molecular biology. We are engaged in the construction of a genetic map based on crosses between 4 genotypes. From three of them, recombinant inbreed lines have been obtained by single seed descent. From the fourth cross between Jemalong and a natural population, we have mapped more than 250 markers (RAPD, AFLP, isozymes, known genes, micro-satellites and morphological markers). This map spans over 980 cM (an average 600kb/cM) in 6 linkage groups (2n=16). Haploid DNA content was measured by flow cytometry to be 0.58pg/1C, that is approximately four times *Arabidopsis* genome size. Level of segregation distortions varies from cross to cross and seem to be related to the genome size of parents[(5)]. This genetic map is a powerful tool for the positional cloning of symbiotic genes and for the breeding of Mediterranean forage legumes as well as for studying evolutionary processes within *Medicago* genus.

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Relationship between Wild and Cultivated gene pools. The particular case of *Medicago sativa* in Spain

Eric Jenczewski, Joëlle Ronfort and Jean-Marie Prosperi
INRA SGAP Montpellier Domaine de Melgueil. 34130 Mauguio. France

Wild and cultivated plants of *Medicago sativa* from Spain are autotetraploid, outcrossing and cross compatible [3,4]. They grow in parapatry in many locations, overlap in flowering periods and share the same pollinators. But, they differ for many morphological traits [1,2]. In this study, we investigated the mechanisms that allow wild populations to maintain their morphological originality. We have examined two hypothesis : Restricted crop-to-wild gene flow or Strong selection pressures exerted against cultivated traits in the wild.

While the level of differentiation at neutral loci only depends on a balance between gene flow and genetic drift, the pattern of variation for quantitative traits may also reflect the effects of selection. Comparing the population structure for neutral and quantitative traits give insight on the relative importance of natural selection and gene flow to explain the morphological differentiation. In 1985 and 1986, one hundred and three natural populations were collected and evaluated in a dense swards experiment. A great variability for both morphological and agronomic traits was detected among populations [1]. For this study, a subset of 15 natural populations and 5 Spanish landraces was chosen to represent the whole range of morphological variability observed. Forty plants per population were individually scored for 13 quantitative traits and 5 allozymic loci.

Combining patterns of population structure suggests [2] :

(1) Evidence for crop to wild gene flow. Natural populations 64 and 147 contained a continuous range of plants with intermediate morphological features between typical wild and cultivated plants. They were neither significantly differentiated from the typical wild populations ($F_{st}=0.009$) nor from the landraces ($F_{st}<0.001$). These populations are likely to be of hybrid origin. This result strongly suggests that gene flow occurred from crop to wild populations.

(2) Strong divergent selection. Natural populations 32 and 66 contained mostly typical wild plants but they were not significantly differentiated from the landrace Aragon for allozymes ($F_{st}<0.064$). These contrasting patterns still provide evidence for gene flow from crop to wild populations, but also suggests that a strong divergent selection eliminated the cultivated traits from natural populations.

(3) Isolated wild populations exist. The remaining natural populations were significantly different from all the landraces with respect to both allozymes and quantitative traits. They are likely to be spatially or temporally isolated from the cultivated populations. The variation of the importance of the gene flow (crop-to-wild) may be due to a combination of factors (agricultural rotations, open man-made habitats).

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Pollen Tube Growth and Fertilization in Tripped and Untripped Flowers of Alfalfa (*Medicago sativa*) Inbred Plants

E.V. Kvasova
Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, 630090, Novosibirsk, 90, Russia.

Tripping is an important process preceding fertilization in alfalfa producing rupture of the stigma surface membrane that prevents pollen tubes from penetration into style of untripped flowers. In field conditions, tripping and cross pollination of alfalfa plants are performed at the same time by the insect pollinators. Autotripping in alfalfa populations occurs very rarely. Application of inbreeding together with selection for autotripping character allows to increase quite rapidly both autotripping level per plant and the number of autotripped plants.
In closed (untripped) flowers of plants of alfalfa populations pollen germinates freely on stigma with intact membrane, and pollen tubes penetrate into ovaries. This was observed in our cytoembryological study. However, ovule fertilization in untripped plants of the population did not occur and, under isolation conditions without flower tripping, seeds did not set. The same was observed in the first three inbred generations: ovules did not fertilize in ovaries of closed flowers in the presence of pollen tubes. A few fertilized ovules in ovaries of untripped flowers first appeared in the fourth inbred generation. Fertilization in untripped flowers was more frequent in the subsequent inbred generations, and it was observed in all plants both of high and low levels of self-fertility after the ninth generation.
Under artificial self-pollination of the population plants a number of pollen tubes and their penetration deep into ovaries are mainly dependent on self-fertile level: the number of pollen tubes is considerably higher in ovaries of high self-fertile plants than in partly self-fertile and self-incompatible plants. Moreover, pollen tubes grow not in all ovaries (their number varies among plants from 26.3 to 85.6%), and the lower was the level of fertility, the smaller was the number of ovaries with pollen tubes. The pattern of pollen tube growth in untripped flowers was the same as in artificially opened (self-pollinated) flowers: the higher was self fertility, the larger was the number of ovaries with pollen tubes and the number of pollen tubes in ovary. Inbreeding affected all characters and processes, including the pattern of pollen tube growth and fertilization. In plants of the population, there was a correlation between the number of ovaries with pollen tubes and self-fertility (r=0.79); in inbred plants, there was no such correlation (r=0.163-0.28). This means that active pollen tube growth cannot be an index of high self-fertility in alfalfa inbred plants. The following was commom to ovaries of untripped flowers of all the inbred plants of different inbred prolongations: 1) free growth of pollen tubes whose number is high enough to provide fertilization of all ovules in ovaries and 2) occurrence of fertilization although less frequent than in ovaries of autotripped flowers.
Prolonged inbreeding led to changes in autotripping time, the patterns of pollen tube growth and fertilization. As a result, we distinguished 4 types of plants differing in fertilization patterns: 1) fertilization in artificially tripped (self- or cross-pollinated) flowers in the absence of autotripping; 2) fertilization in autotripped flowers in the presence of high autotripping level; 3) fertilization in closed (untripped) flowers (rare) and 4) fertilization both in autotripped and in untripped flowers in the presence of opened and closed flowers in the same plants.

Somatic hybridization between *M. sativa* and annual *Medicago*.

Yuko Mizukami*, T. Takamizo, M. Kato*, S. Inami*, M. Kanbe* and I. Houmura***
*** Aichi-Pref. Agri. Res. Ctr., Nagakute, Aichi 480-1193, Japan, ** MAFF. Research Secretariat, Chiyoda, Tokyo 100-5850, Japan**

Some annual Medicago Species have been reported to be the source of useful agronomic traits, for examples resistance to pests and/or diseases. Though they are sexually incompatible with alfalfa, somatic hybridization can produce symmetric and asymmetric hybrids among them. Three hybridizing combinations were examined, M. sativa (2n=32, cv. Rangelander) and M. scutellata(2n=32, cv.Robinson)/ M. rugosa (2n=32, cv.Paragosa)/ M. polymorpha(2n=16, Japanese ecotype). Isolated protoplasts from embryogenic calli of M. sativa and cotyledons of annual Medicago were fused using electrofusion setup (SSH-10,Shimadzu,Japan). Selection of the fused hybrid protoplasts was carried out by means of IOA treatment to M. sativa. After fusion, protoplasts were cultured on KM8P medium using agarose beads method with nurse cells. Individual colonies were proliferated on UM medium and regenerated on half strength SH medium. Regenerated plants were grown in a green house.

More than 9 putative hybrids were obtained from each hybridizing combination. They are all perennial type and their plant morphology was similar to M. sativa, although differentiation were observed among hybrids and hybridizing combinations.

Their chromosome number were ranged from 43 to 56. From the results of Southern blotting analysis by ribosomal DNA (rDNA), all putative hybrids had both the specific bands of M. sativa and those of annual Medicago. Using mitochondorial DNA (mtDNA), a few hybrids had the both bands of parental species and others had those of M. sativa only. In case of chloroplast DNA (cpDNA), the specific bands of annual Medicago were not observed in all hybrids. Therefore, obtained plants through electric fusion were asymmetric somatic hybrids and their organelle composition were the same as a parental form or additive pattern of them. Most hybrids had very low pollen fertility. There were also abnormal morphology of flower.

During four years cultivation, clonal propagation by stem cuttings were carried out. As years passed, their chromosome were deleted and those number became to 32. Some hybrids recovered their fertility. RAPD and RFLP analysis revealed that the hybridity were lost in most of hybrids. It suggested that chimeric or phenotypic unstable plants were caused. It might be given from the chromosome variations ; e. g. deletion, translocation between parental chromosomes and so on.

Further analysis about the chromosomal composition, relative genomic contribution of parents and genomic stability will be needed to stable somatic hybrids/cybrids. In this experiment, only a few hybrids still have kept their hybridity and set some seeds. The analysis using these progeny will be helpful to elucidate those problems.

Distribution of the seed yield in an alfalfa canopy

Christian Huyghe, Eduardo D. Bolanos-Aguilar, Christian Ecalle and Bernadette Julier
INRA, Station díAmélioration des Plantes Fourragères,
F-86600 Lusignan, France

The spatial distribution of the seed yield in an alfalfa canopy was analysed. On one canopy in year 2 of the variety Europe grown at Lusignan, France, with a seed yield of 0.8t/ha, all the number of stems of a sub-plot of 1 m_ were harvested and their individual contributions to seed yield assessed. 260 stems per m_ were present at harvest. Only 68% of these stems contributed to seed yield. The 13% most productive stems produced more than 1.25 g of seeds each and contributed 37% of the total seed yield. The different classes of stems according to their seed production showed little difference in mean seed weight per inflorescence. Their yield mainly varied with the number of inflorescence.

The vertical distribution of the seed yield was analysed on a sample of 30 stems, chosen at harvest as highly productive stems. The different inflorescence-bearing nodes were individually analysed as these nodes also represent a time-scale. The seed production per node declined at the upper nodes both because of a lower frequency of occurrence of the inflorescences and of a lower seed weight per inflorescence. The upper nodes also showed a small decline in the number of seeds per pod and of the mean seed weight. The seed yield per node, the frequency of inflorescence occurrence and the seed weight per inflorescence may be modelled according to their position onto the stems with a mathematical model

y=exp(a x_ + b x + c) with y being the modelled character and x the node position.

When comparing different canopies (locations x sowing years) of the cultivar Europe with contrasted seed yields, it was shown that the variation in the seed production per node was mainly associated to variations in the seed weight per inflorescence and little to the frequency of inflorescence occurrence. The maximum seed weight per inflorescence predicted from the model was highly correlated to the seed weight per inflorescence measured on 30 inflorescences sampled at random in the canopy.

Across 10 environmental conditions and for two cultivars, Europe and Magali, there was a highly significant correlation (r=0.813, P<0.001) between the seed weight per inflorescence and the seed yield.

The physiological mechanisms controlling the variation in the seed weight per inflorescence will have to be investigated.

Variability of regenerated alfalfa plants

Pavol Hauptvogel, Juraj Farago, Katarína Bojnanská, Ján Kraic
(Research Institute of Plant Production), Bratislavská 122, 921 68, Slovak Republic

Alfalfa belongs to the most important forages not only in Slovakia, but also in the whole world. This is confirmed also by the fact that alfalfa is grown on 32 million hectares of arable land. From the aspect of its importance and because of intensive selection and development of local highly productive populations, genetic variability of this crop has declined radically in the last decades. Therefore development of new alfalfa varieties by conventional breeding methods is still more and more difficult. *In vitro* techniques provide an important contribution to the breeding programmes, where classical breeding is insufficient or it fails. But the main assumption of using in vitro techniques for the breeding purposes is regeneration of whole plants from differentiated cells (BINGHAM et al., 1975). In our work, we aimed at the screening of high regeneration ability genotypes in one of the last released Slovak alfalfa varieties Lucia and development of breeding material with high regeneration ability by means of repeated cycles of somatic embryogenesis induction. Moreover, obtained progenies of regenerants were evaluated according to the morphological and agronomical traits. Alfalfa regenerants obtained using *in vitro* culture were analysed cytologically. Our effort was to analyse somatic number of chromosomes of alfalfa regenerants and to verify in this way phenomenon of somaclonal variability occurring in alfalfa cell cultures. In the first cycle of screening alfalfa (*Medicago sativa* L.) genotypes of Lucia variety for regeneration ability, 13 regenerants of R_0 generation have been obtained (KRAIC et al., 1994). In 1996, representative part of the seed S_1 and OP_1 was used for the screening of both progenies of regenerants for *in vitro* regeneration ability (second screening cycle) with the aim to obtain alfalfa population with a higher regeneration ability of genotypes than original variety. At the same time some economic characteristics of clonal as well as S_1 and OP_1 generations of regenerants were tested in field conditions. During the whole cycle of screening for ability to produce somatic embryos the explants were evaluated for the frequency of callus formation, callogenesis intensity, frequency of embryogenic callus production, and frequency of embryogenic genotypes, which were used as the main criteria for selection of embryogenic genotypes. In the field crops, colour of flower, health conditions, average plant height, average number of stems/plant, and average plant weight after each cutting were studied. Results of field experiments have shown a high uniformity of the clones in flower colour. All of the plants were similar to the parental forms and as for the studied morphological traits, no somaclonal variants were found out. Comparison of S_1 and OP_1 populations and clonal progenies under the field conditions pointed out genotype differences in the number of stems/plant, in the average plant weight, as well as average plant height after ascertaining the average of the data obtained in the first harvest year after three cuttings. Inbreeding depression of the studied traits was revealed in the progeny obtained by selfpollination. From the results it follows that changes in chromosome number in alfalfa regenerants ranged from 8 to 28 % of the whole number of analyzed metaphases. The lowest occurrence of metaphases with variance in chromosome number was revealed in regenerant R5, namely 8 %. Up to 20 % of changes in chromosome number were assessed in regenerants R4, R7 (13 %), and R1, R8 (19 %). Other regenerants (besides regenerants that have not been analyzed, R2, R3, R9) had occurrence of aneuploid and polyploid cells in the progeny higher than 20 %. The highest value was recorded in the regenerant R6 (28 %). The results obtained confirm that there exists the possibility of selecting alfalfa population from commercial variety Lucia enriched with the trait of *in vitro* high regeneration ability.

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The Effects of Row Spacing, Inter-row Cultivation and Herbicide Application on Weed Control and Alfalfa Seed Production.

H.A. Loeppky[1], W. May[2] and J.J. Soroka,[3]
Agriculture and Agri-Food Canada, Saskatoon[1,3] and Indian Head,[2], SK,S7N 0X2

Seed purity affects marketability and economic return on alfalfa seed, however, seed stands are relatively non-competitive and weed control options are few. Integration of agronomic practices and herbicides or cultivation could improve weed control and reduce herbicide requirements. Field studies were conducted at 2 sites (Melfort and Tisdale) in NE Saskatchewan to determine the weed control and alfalfa seed production response to two row spacings (22.5 or 67.5cm) and several weed control practices including cultivation or herbicides. Large plots (11m x 73m) of Beaver alfalfa were direct seeded with a Conserva-Pac® no-till drill into wheat stubble early in June, 1994, at a seeding rate of 1.25 kg ha-1. In wide-row plots, Roundup® (880 g.a.i.ha-1) and MCPA Na® (Melfort only, 840 g.a.i.ha-1) were applied between the rows with a shielded sprayer and cultivation was performed between the rows. Cultivation was also tested in narrow-rowed plots; in this case cultivation was not confined to between the rows. Pardner® (280 g.a.i.ha-1, in the seedling year) plus Velpar® (960 g.a.i.ha-1, established), and Pursuit® (50 g.a.i.ha-1) were applied as a blanket application in both narrow and wide rows. Except for the Pardner/Velpar combination, all herbicides were applied each year. Permanent quadrats were established so that alfalfa and weed density could be determined each year, before and after treatments. Visual ratings of weed control were taken each year approximately 4-6 weeks after treatment. Seed yield was determined in both 1995 and 1996.

Alfalfa establishment was not affected by weed control treatments, however, plant density was higher in narrow rows than in wide rows, even though the same seeding rate was used. This could be attributed to less intra-row competition in narrow rows. Seed yield was not affected by row spacing. In 1995, seed yield was not affected by any of the weed control treatments at either location. Weed densities were low due to very dry spring conditions. Roundup, applied between the rows resulted in the lowest yield at Melfort in 1995. In 1996, this treatment significantly reduced seed yield at Tisdale. It also reduced vegetative growth and delayed flowering at this site, which may explain the decrease in seed yield. All other treatments did not effect seed yield at Tisdale and none of the treatments affected seed yield at Melfort in 1996. Canada thistle, one of the most troublesome weeds in alfalfa seed fields, was present at both sites when the alfalfa was planted. None of the treatments significantly changed thistle density at either site, however, good thistle suppression was observed approximately 21 days after application of inter-row Roundup (66% control in 1995, 73% control in 1996), and MCPA Na (79% in both years). Inter-row cultivation provided poor thistle control in 1995, but in 1996 70% suppression was observed at both sites. By the end of the growing season, however, the Canada thistle had completely recovered from these treatments.

Alfalfa's Influence on Rotation and Soil Physical Properties

Lynn D. Hoffman and W. Scott Harkcom
Pennsylvania State University

Corn following alfalfa consistently yielded twenty-one bushels more than corn following corn in a crop rotation study now in its thirtieth year at Penn State.

The presence of alfalfa in this study affects the economics of crop production. Compared to the continuous corn or corn-soybean rotation, it favorably affects soil physical properties. Decreased soil pentitrometer resistance and better water infiltration are consistent advantages found in the alfalfa plots compared to other rotations. A relatively new evaluation for soil quality ó Phosphataise enzyme-measuring the soil microbial activity suggests better soil quality and a "healthier soil" in the alfalfa rotation. This measurement has promise to better explain the prevailing changes in conditions influencing the consistently higher corn yields following alfalfa. Corn yields of second and third year corn following alfalfa usually become consistent with continuous corn yields. This has economic implications to the seed industry as shorter rotations would increase frequency of alfalfa establishment and increase seed sales. We know the value of alfalfa as a forage crop. Data from this study validate its importance in farm management decisions to increase net returns in the overall farm enterprise.

Evaluation of Grazing-Tolerant Alfalfa Cultivars Across Diverse Environments

A.Singh[1,2], W. P. McCaughey[1], and S. R. Smith, Jr.[2]
[1]Agriculture and Agri-Food Canada, Brandon Research Centre, P.O. Box 1000A, Brandon, MB, R7A-5Y3, [2]University of Manitoba, Plant Science Department, Winnipeg, MB, R3T-2N2

Information on alfalfa cultivar performance under grazing in the northern Great Plains is very limited. Climatic and edaphic conditions of the northern Great Plains are varied, and their influence on the persistence and productivity of alfalfa pastures are also varied. At present, a standard evaluation procedure to assess grazing tolerance has been accepted by the North American Alfalfa Improvement Conference (Bouton and Smith 1996), however its applicability to the northern Great Plains region remains unanswered. Although the standard protocol is effective at "sorting out" cultivar persistence under continuous stocking, it does not specifically address grazing tolerance under rotational stocking systems or the inclusion of a grass component, both of which are likely preferred management options for western producers. A preliminary study conducted near Brandon, MB confirmed that continuous stocking as an effective management system for isolating potential sources of germplasm for breeding environmentally adapted, grazing tolerant cultivars. The same study revealed an interrelationship between winterhardiness and grazing tolerance. Observed stand losses may have been attributed to winterkill or grazing stress, or quite possibly a combination of the two. This highlights the need for grazing tolerant cultivars in the northern Great Plains to be both persistent under grazing, as well as exhibiting a high degree of winterhardiness, while maintaining high yields.

A comprehensive grazing research project is currently addressing the development of grazing tolerant alfalfa cultivars that are well adapted to the diverse environments of northern Great Plains. A collection of 23 alfalfa (*Medicago* spp.) Cultivars and experimental lines is being evaluated in a 3 year grazing experiment to assess persistence in pure stands and in mixture with meadow bromegrass (*Bromus biebersteinii* Roem & Schult.) Under both continuous and rotational stocking systems. In 1996, experimental sites were established at Brandon, MB; Swift Current, SK; and Lethbridge, AB, and in 1997 at Mandan, ND. The different soil types and extremes in moisture and temperature will provide adequate comparative data for evaluation across varying environments. Alfalfa cultivars have been traditionally categorized as hay-type (tap-rooted) or pasture-type (creeping-rooted), but many new experimentals are classified as dual-purpose cultivars, and exhibit other characteristics such as improved disease and insect resistance. In an effort to determine the most efficient and accurate method for assessing grazing tolerance in alfalfa a detailed protocol is being used for evaluation, which includes winter survival, spring vigour, percent alfalfa (within stand and row), percent grass, biomass yield, and botanical composition. This research is being jointly funded by the United Grain Growers: Proven Seed Division, ABI, Agriculture and Agri-Food Canada, and the University of Manitoba.

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The evolving role of lucerne in Australia

William Bellotti[1], Geoff C. Auricht[2], Rex Williams[3] and Michael J. Hill[4]
[1] The University of Adelaide, Roseworthy, South Australia, 5371, [2] South Australian Research & Development Institute, Adelaide, SA, 5001, [3]New South Wales Agriculture, Tamworth, New South Wales, 2340, [4]CSIRO Animal Production, Perth, Western Australia, 6014

The area of lucerne (irrigated and dryland) in Australia is expanding rapidly. In the past decade, the official (Bureau of Statistics) estimates of lucerne area have more than doubled, from 351,000 ha (1982/83) to 772,000 ha (1993/94). These figures underestimate the total area of lucerne containing pastures as they include only ëpureí lucerne stands. Other area estimates, based on surveys of agronomists (Pearson et al., 1997) and bioclimatic modelling (Hill, 1996) indicate further large increases are entirely feasible.

Two driving forces behind the current expansion include:

1. Use of lucerne for grazing, forage conservation, seed production, and for rotational benefits (soil nitrogen, soil structure) in cropping systems is highly profitable.

2. Lucerne has several advantages for addressing a growing number of land degradation issues such as; dryland salinity (lucerne has high water use compared to annual plants), soil acidification (lucerne can reduce the rate of soil acidification through recovery of leached soil nitrate), and herbicide resistant weed populations (lucerne provides the basis for integrated weed management).

To better fulfil these new roles in developing more sustainable farming systems, there needs to be a shift in emphasis in Australian lucerne improvement programs. Traditional lucerne breeding objectives have included; disease resistance (*Phytophthora* root rot, *Colletotrichum* crown rot, stem nematode); pest resistance (spotted alfalfa aphid, blue green aphid); high herbage production, long-term persistence (> 7 years); across a range of dormancy types (winter dormant to highly winter active). More recently, ease of establishment and removal have been included as desirable traits for inclusion of lucerne leys in cropping systems.

New breeding objectives, in addition to the traditional objectives listed above, would be related to enhancing the ability of lucerne to perform its sustainability function and to expand lucerne adaptation onto less favourable soil types. Possible objectives are suggested in the table.

Attribute	Genetic variation	Reference
high water use	?	

rapid root elongation	yes	Meyers et al., 1996
water logging tolerance	?	
salinity tolerance	yes	Smith et al., 1994
soil acidity tolerance	yes	DallíAgnol et al., 1996
soil nitrate depletion	yes	Lamb et al., 1995

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The production and water-use from dryland alfalfa in Western Australia

R.A.Latta, L.J.Blacklow and P.S.Cocks
Agriculture Western Australia, Katanning, Western Australia, 6317.

A dryland alfalfa (*Medicago sativa)* production system is being evaluated in the wheat-belt of Western Australia as an alternative to annual pasture leys. This is supported by the need to increase water-use to arrest rising water tables and associated land salinisation. Historically alfalfa has been restricted in WA due to acidic soils and the Mediterranean climate. However, the identification of more effective *rhizobial* inoculants (Evans and Howieson, 1992) and winter active cultivars has improved alfalfa productivity and persistence. Two experiments, (1) alfalfa versus sub-clover (*trifolium subterranean)* and (2) versus annual medic (*Medicago polymorpha*) were conducted on duplex soils, with pH's of 5.0 and 5.8 ($CaCl_2$). Comparative pasture growth rates and soil water assessments, with a neutron moisture meter, were measured. Average annual rain is 400 and 350mm (70% occurring April to October) with an annual pan evaporation of 1600 and 2000mm. Mean maximum temperature in summer 32°C, minimum in winter 6°C. Alfalfa plant densities were 30 and 50m^{-2}, the sub-clover >500m^{-2} in both years, the annual medic 50m^{-2} in first year, >300 in second year. Less than 100 plants/m^{-2} of volunteer annual grass made up the remainder of the pasture. The alfalfa treatment maintained or increased bio-mass production (Table 1) and reduced water stored in the 0-150 cm soil profile (Table 2).

Table 1. Alfalfa and annual pasture biomass production (kgDM/ha)

	Experiment 1		Experiment 2	
	Summer	Winter	Summer	Winter
Alfalfa	2000	4000	2000	5000
Sub-clover(Exp1) Annual medic (Exp2)	1000	5000	200	4800

Table 2. Soil water under alfalfa and annual pasture (mm H_20 0-150cm)

	Experiment 1		Experiment 2	
	Summer	Winter	Summer	Winter
Alfalfa	130	230	160	210
Sub-clover(Exp1) Annual medic (Exp2)	230	280	220	240

Eastern Australian studies have found that alfalfa will reduce stored soil water by extending the pasture growing season into the late spring and summer and may also increase pasture production (Crawford and Macfarlane, 1995). The results from this study suggest similar agronomic and environmental benefits may be achievable in Western Australia. The Australian Grains Research and Development Corporation provided financial support to this project.

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The status and perspective of alfalfa production industriation in China

Yufa Zhang
Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100094. China

Alfalfa often is called "Queen of the Forages". It has been planted over 2000 years in China, and has made a great contribution to the development of agriculture and animal husbandry of China. Alfalfa growth area in China is about 1.33 million ha, mainly distributed in the north part of China.

Chinaís animal husbandry industry has been growing fast and stablely in the last two decades, with a increasing rate over 10% for each year. We are facing a big problem - shortage of supplying for feed cereal. To develop alfalfa production is one of most important ways to resolve this probem . There is a good opportunity for Chinaís alfalfa growth. Now the situation of Chinaís alfalfa growth is excellent. The characteristics are the following six aspects. 1.To establish large alfalfa base, and to form integrated system, combining alfalfa growth, animal raising, processing and marketing. China Animal Huabandry Group is carrying on a project, which includes planting 10 thousends ha alfalfa , raising 10 thousands cattles and building beef process plant. More and more enterprises, including national, cooperative, and individules. 2.To develop and market alfalfa hay , meanwhile to develop dairy industry around urban areas, such as Tianjing, Shijiazhuang, capital city of Heibei Provence. 3. To provide alfalfa harvest and processs equipment, including the ones imported from forgein countries and also the ones manufactured domesticaly. The first alfalfa cubes plant, designed 60 thousands tons process capability per year, was built in Xinjiang in 1996. The process equipment was introduced from USA. More plants are needed to be built. 4. To carry on integrated alfalfa production research program, for instance, Japen government invested $ 4 million in Hebei Provience to explore salt land in east of China. 5 To seek forein companies investment in alfalfa production. Such as some companies, including United States, Canada, Australis, began to enter into China market.

It is estimeted that alfalfa land will be increased to 3 - 4 million ha in 2010. There is a great potential in alfalfa harvest and process equipment market ,which is about $1000 - 1300 million . A lot of alfalfa research programs need to be conducted in the next Century. Chinaís alfalfa industrilization still has a long way to go, but we believe that it will be developed rapidly.

The Effect of Physiology and Production of Dairy Cattle When Fed Alfalfa Hay

Yuegau Hu, Kemin Su, Juigang Duan, Yue Chen
China Agricultural University, Beijing, 100094, People's Republic of China

Small dairy farms are going to be the main model in the China dairy production system. The ration ingredient is composed of concentrate feed and crop stalk. However, there are two kinds of problems with this system, i.e. low milk output and weak unhealthy cows. In order to seek the way to solve this problem, one experiment, using alfalfa hay in place of crop stalk (1kg, 2 kg, 3kg daily), has been done in Mingyi Yang and Yucai Yang dairy farm, Dongshuangtang village, Tianjing. Forty cows were selected, and divided into 4 treatments. The experiment lasted 40 days. Ten head days was arranged as primary experiment stage with the rest of it as the formal experiment stage. The experiment was conducted from February 10 - March 20, 1995. The experiment result is as follows: 1) Using alfalfa hay as an alternative to crop stalk in the diet can clearly raise milk quantity. Each treatment increased milk daily, with the baseline being 0.078, 0.268, 1.681, 1.509kg/h , respectively. The No. 3 treatment is the highest. Per kilogram alfalfa hay alternative can produce 0.809kg more milk. While No. 2 and No. 4 were 0.268kg. Increasing quantity had a larger effect on the cows with high lactating level than low lactating level; 2) There are not significant deference on the milk specific gravity, milk fat content, as well as milk dry matter content among the treatments during the experiment; 3) There are significant differences on the serum selenium and the activity of glutathion peroxidase in whole blood (GSH-PX) among the 4 treatments. The GSH PX activity is statistically different after 7 days from the beginning of the experiment until the end of experiment, while the serum selenium indicate significant differences on 14 days after the experiment began, and keeps the same state until the end. There are no statistical difference among the treatments on the blood chemistry (Ca, P, Mg, Zn), the activity of serum enzymology (AST, ALT, GGTP, ALP, LDH, HBDH), the serum protein (TP, ALB, GLOB, ALB), etc.

The Experiment Treatments and Results

Program/Treatments	1	2	3	4
Alfalfa Hay Fed	0kg/d	1	2	3
Ave. Rep. Times	3times	35829	3	35829
Ave. Lact. Month	3.6months	4	4.5	3.7
Lact. Output (Primary)	19.60kg/d/h	17.49	21.55	19.94
Lact. Output (Exper.)	19.61kg/d/h	17.76	22.77	21.45
Total Incr. Output	24.00kg	80.4	485.4	452.7
Ave. Incr. Output	0.078kg/d/h	0.268	1.618	1.509
Milk Spec. Gravity	1.031	1.03	1.031	1.029
Milk Fat Content	3.035%	3.005	3.075	3.02
Dry Matter Content	11.815%	11.739	11.739	11.689
Serum Selenium	0.046 mg/kg	0.055	0.082	0.089
GSH-PX Activity	8.69IU/ml	9.59	19.49	18.87

Measurement of Rumen Undegraded Protein by Near Infrared Spectroscopy

Dan Undersander and Pat Hoffman
Agronomy and Dairy Science Departments, University of Wisconsin
1575 Linden Drive, Madison, WI 53706

Rumen Undegraded Protein (RUP) or by-pass protein provides certain amino acids to the high producing dairy animals in quantities not available from microbial protein. Therefore measurement of this constituent is critical to balancing rations and maximizing milk production. The problem is that the actual RUP of a forage is dependent on the forage, animal, and level of feed intake (or rate of passage). Numerous chemical and enzymatic techniques have been developed but all show significant deviations from in situ measurements. However, *in situ* measurements are not feasible for commercial measurement of RUP due to analysis time and cost of animal maintenance and measuring microbial protein in the sample after *in situ* digestion. Further, *in situ* results from a single animal will vary greatly from in situ results of other animals.

We have developed a Near Infrared Reflectance Spectroscopy (NIRS) equation to estimate RUP of a legume or grass-legume silage based on 24-hour *in situ* digestion of the sample. Silage samples for equation development were from those submitted to forage testing laboratories. They were oven-dried and ground through 1 mm cyclone mill. A technique was developed to 'standardize' cows and all samples were digested in duplicate in 8 dairy cows. An NIRS equation was developed to measure microbial proteins with a reference procedure based on purine content (Zinne and Owens with modification by Aharoni and

Tagari (1991). Crude protein was determined on the digested residue by Kjeldahl. Samples were read on Perstorp Model 6500 before digestion (for equation development) and after digestion (to determine microbial protein in the sample). The equation was developed using ISI version 4.0 software. The RSQ of the equation was 0.84 and SEC was 1.55.

Results of the *in situ* measurements also indicated the problems of other common measurements of RUP. The correlation (RSQ) of *in situ* measurements with acid detergent insoluble nitrogen, neutral detergent insoluble nitrogen, and soluble protein were all low at 0.25, 0.30, and 0.50, respectively.

GENETIC IMPROVEMENT OF ALFALFA TO CONSERVE WATER

D. CLAYPOOL[1], R. DELANEY[1], R. DITTERLINE[2] AND R. LOCKERMAN[2]
[1]University of Wyoming and [2]Montana State University

Irrigated alfalfa (Medicago sativa L.) is a high user of water and has received limited research to improve its water-use efficiency (WUE). Alfalfa is grown on approximately one-half of the cultivated land and has the highest consumptive water use of all crops in Wyoming. Alfalfa lines were developed which expressed lighter colored and larger leaves. The water relations and productivity of these lines were evaluated under a series of soil water levels in a controlled environment and under field environments in Wyoming and Montana. In the controlled environment the WUE of the pale and dark leaf genetic lines were 1.47 and 1.22 g dry forage kg^{-1} water, respectively, when averaged across seven soil moisture levels. Large-leaved alfalfa plants produced 1.43 and small-leaved 1.29 g dry forage kg^{-1} water. Leaf transpiration per unit leaf area of pale leaves in irrigated field studies ranged from 12 to 17% lower than dark (normal) leaves. Transpiration of large leaves was 10 to 16% lower than small (normal) leaves. In field studies, the forage yield of pale leaves was 25 to 29% higher than dark leaves. The yield of large-leaved plants was 17 to 22% higher than small-leaved. These studies indicate the WUE of alfalfa can be improved through plant breeding to conserve water in the western U.S.

Characterisation of alfalfa populations derived from selection for cold tolerance

A. Claessens*, P. Nadeau[2], Y. Castonguay[2], G. Allard[1] and R. Michaud[2]
Univ. Laval[1] and Agriculture and Agri-food Canada[2], Sainte-Foy, Quebec.

Alfalfa (*Medicago sativa* L.) is the most commonly grown forage legume in North America due to its excellent agronomic qualities. However, its lack of persistence under severe winter conditions reduced its utilisation in the eastern provinces of Canada. Cold tolerance has been shown to be the most important factor in winter survival. Selection for cold tolerance is difficult due to the complexity of field evaluation. A method of selection performed under environmentally-controlled conditions has been used for the identification of genotypes having superior cold tolerance.

Briefly, 1500 plants (cv Apica) were cold hardened for 2 weeks at 2°C and for 2 additional weeks at -2°C before they were subjected to three successive freezing stresses. The freezing temperatures were -10°C for the first and the second stress and -12°C for the third. Plants were selected on the basis of their cold tolerance and the vigour of their regrowth, and intercrossed to produce a first cycle population potentially more cold tolerant (CT1). Similarly a second cycle of selection was performed using CT1 to produce a CT2 population. The original population (Apica) and the two populations derived from our selection (CT1 and CT2) were evaluated for their cold tolerance (LT_{50}), cryoprotective sugar accumulation and for the expression of two genes (*msa*CIA and *msa*CIC) regulated by cold temperature (Castonguay *et al.*, 1995; Castonguay *et al.*, 1997).

The results of this study indicated that alfalfa cold tolerance (LT_{50}) and regrowth after exposure to cold temperatures could be improve by a selection based on successive freezing stresses. Cold tolerance progressively increased at each cycle of selection from -16.9°C for Apica to -17.4°C for CT1 and -19.1°C for CT2. A repercussion of this selection was the significant increase of the cryoprotective sugars, stachyose and raffinose, at the crown level. The CT1 and CT2 populations selected for superior cold tolerance accumulated significantly higher levels of those two sugars in February, comparatively to the original population (stachyose : 12.45, 14.21 and 16.94 mg g^{-1} DW, raffinose : 15.34, 18.70 and 21.73 mg g^{-1} DW for Apica, CT1 and CT2 respectively). The selection did not modify the expression of the *msa*CIA and *msa*CIC genes. Our results indicated that the use of successive freezing stresses is an efficient approach for the development of alfalfa populations with superior cold tolerance.

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Freezing Tolerance Mechanisms of Suspension Cells Derived from Alfalfa Cultivars Differing in Fall Dormancy.

N.E. Kalengamaliro, J.A. Gana, S.M. Cunningham, and J.J. Volenec
Department of Agronomy, Purdue University, West Lafayette, IN 47907-1150

A major factor limiting persistence of alfalfa in the Northern US is poor winter hardiness. Cold acclimation increases tolerance of fall dormant alfalfa to subsequent freezing temperatures. Our objective was to determine if suspension cells derived from cultivars exhibiting contrasting fall dormancy reaction also differed in freezing tolerance. Suspension cells derived from Pioneer 5262 (fall dormant, winter hardy) and Pioneer 5929 (nondormant, nonhardy) were acclimated at 2ºC for 14 d, then frozen for 30 min at 0, -5, -10, -15, -20, and -25ºC. Cells were slowly thawed and cell viability determined using 2,3,5-triphenyl tetrazolium chloride (TTC). Acclimated cells of 5262 survived to -25ºC whereas cells of 5929 died at temperatures below -5ºC (Fig. 1)

Sugar concentrations increased 31-fold during cold acclimation of 5262 when compared to cold acclimated 5929 cells. Starch concentrations of acclimated 5262 cells also were greater than those of acclimated 5929 cells. High levels of α-amylase activity occurred in cells derived from 5929 irrespective of temperature. In contrast, cold acclimation at 2ºC reduced α-amylase activity of 5262 cells to 1/4th that observed at 27ºC, and may have permitted starch to accumulate in cells grown at 2ºC. Cold acclimation reduced soluble protein concentrations of 5262 cells, while it had no effect on protein concentrations of 5929 cells. Several polypeptides disappeared during cold acclimation of 5262 cells, whereas no change in protein composition was observed during cold acclimation of 5929 cells. Cold acclimation and freezing tolerance of these cell lines mimicked winter hardiness responses of the cultivars from which the cells were derived.

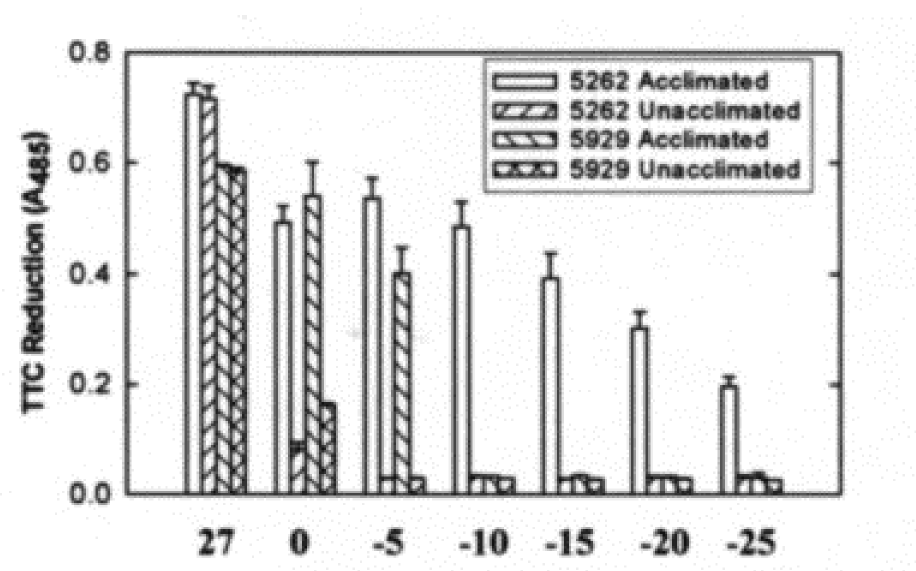

Figure 1. Freezing tolerance of alfalfa cells measured using TTC reduction (proportional to respiratory activity). 5262 is a fall dormant, winter hardy cultivar, whereas 5929 is a nondormant,

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Genetic Correlations Between Yield and Water-Use Efficiency Traits in Water Stressed Alfalfa

Ian M. Ray, M. Shaun Townsend, and Cherokee M. Muncey
New Mexico State University, Las Cruces, NM 88003

Inadequate supplies of surface water limit forage production in the southern Great Plains and western United States. Alfalfa

(*Medicago sativa* L.) production in this region may benefit from improvements in water-use efficiency (WUE), the amount of forage and root biomass produced per unit of water transpired. If benefits from improved WUE are to be realized, correlations between important agronomic traits and key physiological traits associated with WUE must be determined under water-limited conditions. This study characterized genetic correlations among carbon isotope discrimination (D), canopy temperature, ash content, dry matter yield, forage maturity, and leaf-to-stem ratio (LSR) in alfalfa grown under suboptimum irrigation. Heritabilities of the traits on a progeny mean basis were also determined. Thirty semidormant half-sib families were evaluated in seeded plots that were irrigated every 30 d during 2 yr near Las Cruces, NM. Carbon isotope discrimination was negatively correlated with canopy temperature and ash content. An increase in dry matter yield was associated with higher D, lower canopy temperatures, low ash content, taller shoots, earlier maturity, and reduced LSR. Carbon isotope discrimination, ash content, and yield were moderately heritable ($h2$=0.40-0.56) indicating that these traits could be altered through breeding and selection. The positive relationship between D and shoot yield suggests that germplasms should be evaluated for both D and yield when characterizing alfalfa for high WUE in order to minimize potential yield reductions that may result from selection based only on D. Moderate correlations between D and canopy temperature or ash content indicated that neither trait provided highly reliable estimates of D in water-stressed alfalfa.

New Salt Tolerant Cultivar: "Zhongmu No.l" Alfalfa

[1]Yang Oingchuan [2]Sun Yan [3]Geng Huazhu [1,3](Institute of Animal Science, CAAS, Beijing,100094) [2](Beijing Agricultural College, Agronomy Department, Beijing, 102206)

The new salt tolerant cultivar of alfalfa (*Medicago sativa* L. cv. "Zhongmu No.l") was registered by Chinese Herbage Cultivar Registration Board in 1997. It was bred by 4 generation mass selection in saline land with 0.3-0.5% NaCl during 1988-1994, Involving 4 parents: Baoding(Medicago sativa L. cv. Baoding), Nanpi(Medicago sativa L. cv. Nanpi), Rs(Medicago sativa L. cv. Rs) and Bilu(Medicago sativa L. cv. Bilu).

Description of zhongmu No.l: Plants erect, 50-lOOcm high, distinct main root, more lateral roots, leaf color is light green, flower color is from purple to light purple, florescences recamous, seedpods spiral 2-3 circles, seed kidneylike and yellow, Thousand seed weight 1.82-1.9g. growing luxuriantly, good salt tolerant in seedling period. It can be cut 3-4 times per year at huanghuai hai plain and Bohai Bay in China.

The cultivars comparative test and production test were carried out at Zhongjie friendly farm in Hebei province, Dezhou farm in Shandong province, Wudi county in Shandong provine and Lanzhou city in Gansu provine during 1995-1997. At Dezhou test location, The results show (see tablel): Zhongmu No.1 was sown on September 7,1994 in 0.25-0.83% NaCl saline land. The total yield was 5137.5kg/ha in 1995(Cut two times), the total yield and mean plant height exceed Baoding by 13.4% and 8.7 respectively in 1995. The total yield was 5424kg/ha in 1996(cut two times), the total yield and mean plant height exceed Baoding by 11.1% and 5.7% respectively.The total yield was 8602.5kg/ha in 1997(Cut three times), the total yield and mean plant height exceed Baoding by 13% and 4.6% respectively in 1997. At wudi location, It was sown in 0.48% NaCl saline field in august 1995, The total yield was 4455kg/ha(Cut two times), exceeds Baoding, Bilu,Longdong by 35.5%,35.7%,76.8% respectively in 1997. At Lanzhou location, It was sown in 0.21-0.74% Na_2SO_4 field in 1995, It's total yield was 13722kg/ha in 1997, exceeds local Longzhong alfalfa by 18%.

"Zhongmu No.l" alfalfa is adapted for use in northern China for 0.25-0.83% NaCl or Na_2SO_4 saline land.

Table 1. The yield and the plant height of Zhongmu No.1 in 0.25-0.83% NaCl field at Dezhou test location

					Mean exceed control cultivar by (%)	
			Yield	Plant Height		
Year	Stubble	Cultivar	(kg/ha)	(cm)	(Yield)	(Plant height)
	First cut	ZhongmuNo.1	2935.5	51.8		
1995		Baoding(ck)	2554.5	46.6	14.9	11.1
	Second cut	ZhongmuNo.1	2202	52.8		

		Baoding(ck)	1968	49.7	11.9	6.2
	First cut	ZhongmuNo.l	3024	50.33		
1996		Baoding(ck)	2707.5	48.20	11.71	4.42
	Second cut	ZhongmuNo.l	2400	53.6		
		Baoding(ck)	2175	50.2	10.57	6.89
	First cut	ZhongmuNo.l	4531.5	62.2		
		Baoding(ck)	3973.5	57.9	14.0	7.4
1997	Second cut	ZhongmuNo.1	2098.5	60.2		
		Baoding(ck)	1867.5	57.6	12.4	4.5
	Third cut	ZhongmuNo.1	1972.5	61.9		
		Baoding(ck)	1750.5	58.5	12.7	6.4

Evaluation of Different Alfalfa (*Medicago sativa L.sensu lato)* Varieties Under Different Concentrations of NaCl During Germination Stage

Manal M. Hefny [1] and R. Doliñski [2]
[1]Agron.Dept.,Fac. of Agriculture,Suez Canal Univ., Ismailia,Egypt. [2]Institute of Plant Breeding and Genetics,Agric.Univ.,Lublin,Poland.

Alfalfa(*Medicago sativa*L.)is the predominant perennial legume species used as forage crop or pastures,it is grown and produced largely in irrigated arid and semiarid regions.Such regions are affected seriously by salinity, which results in decreasing the area of arable lands.Salinity effects may vary depending upon the growth stage at the time of stress,meaning that, salt tolerance at germination is not consistently related to tolerance during emergence, vegetative growth, flowering or fruiting (Shannon, 1985). While, maximum genetic gain in salt tolerance during germination of alfalfa seeds may only be achieved by selection in solutions containing more than one salt(Rumbaugh,et al.,1993),selection in NaCl solutions may account for most of the potential gain.Also,germination percentage decreased and germination time increased with increasing salinity(Lombardo and Saladino, 1997).Because saline soils and water extremely limit the productivity of crop and pasture lands in semiarid and arid environments,the development of cultivars with the ability to germinate under high salt stress would be useful in such soils. Therefore, fourteen alfalfa (*Midicago sativa L. sensu lato*)varieties,five Polish,four Egyptian,four Hollandian and one American were evaluated under nine concentrations of NaCl at Institute of Plant Breeding and Genetics,Agricultural University,Lublin,Poland.Results showed significant differences among varieties across NaCl concentrations for the germination characters, indicating that, such characters were influenced by both varieties and NaCl levels.In addition,the Egyptian variety ISM-94, was the best variety among the genetic materials,since,it represented the highest IC50 value (229 mM),mean germination percent(64.60%),mean adjusted germination percent(73.40%),the longest radicle and hypocotyl(36.10mm and 25.90mm,respectively)and more rapid germination.Also,highly significant (P(0.01)differences were found among the mean IC50 values of the centers of diversity and among the subspecies,as anticipated,germplasm from the more arid Indian and African centers were better able to tolerate NaCl during germination(Rumbaugh and Pendery,1990).It is obvious that,the concentration 120 mM was the critical one which above it,an observed reduction in the characters mean has been occurred. The moderate value of heritability with reasonable genotypic variance which has been obtained,make selection and improvement of salt tolerance during germination possible.

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Prediction of alfalfa tolerance to soil acidity and aluminium

Pavol Hauptvogel
(Research Institute of Plant Production), Bratislavsk 122, 921 68, Slovak Republic

Current negative effects of abiotic factors are one of the main world-wide problem in ensuring agricultural production and people nutrition. From the whole acreage of agricultural land in Slovakia even 28 %, i.e. 700 000 ha, is the land with acid soil reaction below pH 5.5. Our research was aimed at selection of alfalfa genotypes tolerant to soil acidity and increased concentration of free aluminium ions. Aluminium belongs to the main parts of the soil, where it is released from aluminium-silicates. In the soil, aluminium is present in the form of hydroxides with different stability. Stability of aluminium compounds is determined especially by environment acidity, i.e. that with increasing acidity also the content of free aluminium ions, which can react with other ions, is also increasing. High concentration of aluminium dissolved in the soil is the main factor limiting the yield of cultivated crops. In our experiments for evaluation of alfalfa genotype tolerance to low pH and higher concentration of aluminium ions, a modified method for testing the effect of irrigation on alfalfa plants was applied. In the stage of 5th leaflet we started to apply an acid rain watering with addition of aluminium salts and nutrients in the sand cultures on evaluated alfalfa genotypes. For artificial acid aluminium watering, the mixture of solution of sulphur acid (H_2SO_4) and nitrogen acid (HNO_3) with pH 4.0 ($\pm$ 0.2) and with addition of aluminium chloride ($AlCl_3.6H_2O$) in 20 ppm concentration was used. This solution, together with nutritious Knopp? s nitrogen-free solution, was applied 10 times. On the basis of statistical evaluation by variance analysis, a highly significant effect of substrates in selected traits - root weight, plant weight, and number of rhizobium nodules per 1 plant was found out in evaluated experiments. From the aspect of varieties, highly significant interaction between the tested varieties and substrates was revealed in nodule number per plant. Varieties were characterized by a very high variability in nodule number per plant. Evaluating the plant height, the highest average plant height was found in the standard substrate (33.32 cm) and the lowest one in the silicic substrate (22.99 cm). Highly significant correlation between the evaluated traits within particular substrates was also found in the experiment. Strong correlation was between the root weight and plant weight in the sand substrate (0.77++), silicic substrate (0.80++), and soil substrate (0.80++). Biological material, which was tested in individual substrates, was also used for preselection of the plants tolerant to the given negative abiotic factors. Selected tolerant plants of individual varieties were used in the following year for evaluation in the field conditions with the aim of further utilization in hybridization programme and further assessment of the level of tolerance to low pH and Al^{3+} ions in alfalfa varieties and populations.

Medic grazing/small grain rotation: Results from a SARE farmer grant.

J. Alger, J.R. Sims, D.N. Oien. Timeless Seeds, Inc.; Conrad, MT

The objective of our SARE grant project was to determine whether it was more economically feasible to rotate a wheat or barley field with black medic rather than continuously recropping that field with another small grain crop. Our group wanted to know if legume grazing would be a sustainable alternative to traditional dryland farming methods. Prior to beginning this project, the Alger Ranch would seed a given field to crop for three years and fallow for one year. With this project, Alger alternately harvested a small grain crop one year, and grazed black medic the following year.

In this study, we had two test fields that we seeded to black medic, a 35 acre field and a 28 acre field (fields 35 and 28). These two test fields were seeded to medic in alternate years, giving us, in any given year, one field for harvesting wheat or barley and one for grazing medic. We compared these two test fields to a 16 acre control strip (field 16), which was recropped every year with no black medic.

Conclusion: Approximately 10 years of medic rotations are required to achieve higher organic matter levels in the soil. It is difficult to put a value on the higher organic matter in the soil. We noticed that instead of depleting the soil, the medics are regenerating the fields by adding orgainic matter. This higher organic matter has helped improve soil structure and aeration of the soil. Higher orgainic matter has also increased the amount of natural nitrogen available to the plant which has improved our protein levels on our wheat by 1%. Higher organic matter has also improved the cation exchange capacity of the soil. For every one percent increase in organic matter, the soil will now absorb and retain another inch of moisture. Finally, as the organic matter has increased the pH level in the soil decreased from 7.9 in 1990 to 7.2 in 1997.

Associations Among Forage Quality Traits, Disease Resistance, and Vigor in Alfalfa.

Carlos E. L. Fonseca, D.R Viands, J.L. Hansen, A.N.Pell, J.E. Miller-Garvin,and E.M. Thomas
Dept. of Plant Breeding, Cornell University, Ithaca, NY 14850

Much of the alfalfa breeding efforts in the USA aim at improving forage quality and multiple disease resistance. Little is known about the genetic relationship among diseases, quality traits, persistence, and vigor in alfalfa. We determined the associations among resistances to anthracnose (ANT), bacterial wilt (BW), Fusarium wilt (FW), Phytophthora root-rot (PRR), and Verticillium wilt (VW); concentrations of acid detergent fiber (ADF), acid detergent lignin (ADL), crude protein (CP), true *in vitro* dry matter digestibility (IVDMD), neutral detergent fiber (NDF), and neutral detergent-soluble fiber (NDSF); persistence; and vigor. Plants were scored for disease symptoms according to the Standard tests to characterize alfalfa cultivars (Fox et al., 1991). Simple (r_S), phenotypic (r_P), and additive genetic (r_G) correlation coefficients were estimated for all possible pairs of traits from half-sib (HS) progeny tests of alfalfa populations NY9505 and NY9515. Both plant populations are of moderate fall dormency (rating 4) and are adapted to northern USA and eastern Canada.

Significant differences among HS families were detected for all traits, except for persistence. Resistances to BW and FW were significantly associated in NY9505 ($r_G = 0.61 \pm 0.12$). In both populations, NDSF (mostly pectin in alfalfa), was significantly associated with ADF ($r_G = -0.67 \pm 0.11$ and -0.86 ± 0.10), ADL ($r_G = -0.76 \pm 0.11$ and -0.86 ± 0.10), IVDMD ($r_G = 0.53 \pm 0.15$ and 0.71 ± 0.12), and NDF ($r_G = -0.71 \pm 0.10$ and -0.88 ± 0.09). Vigor was significantly associated with ADF ($r_G = 0.50 \pm 0.16$), ADL ($r_G = 0.55 \pm 0.18$), CP ($r_G = -0.42 \pm 0.19$), IVDMD ($r_G = -0.65 \pm 0.17$), and NDF ($r_G = 0.53 \pm 0.16$) in NY9515. Significant association was found between FW and CP ($r_P = -0.22 \pm 0.11$), NDSF ($r_P = 0.25 \pm 0.11$), and vigor ($r_S = -0.17 \pm 0.07$) in NY9505, and between ANT and both NDF ($r_G = 0.30 \pm 0.14$) and vigor ($r_G = 0.31 \pm 0.15$) in NY9515. We conclude that selection for BW resistance in some populations may have a positive impact on FW resistance. Selection for higher NDSF concentrations may decrease the concentrations of the other cell-wall fiber components and increase IVDMD. Selection for lower fiber and/or higher CP concentrations may decrease vigor. Significant correlations between quality traits, disease resistances, and vigor were not of sufficient magnitude to adversely affect the improvement of these traits. Response to direct selection may be effective for both populations, but other related traits may need to be monitored for indirect response from selection.

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Response to Four Cycles of Selection for Low Initial Rate of Digestion in Alfalfa.

B. E. Coulman, C. Duncan and B.P. Goplen
AAFC, Saskatoon Research Centre, Saskatoon, SK. S7N 0X2

Between 1979 and 1971 four cycles of recurrent selection for low initial rate of digestion (LIRD) were carried out in a population of alfalfa, composed of the cultivars Beaver, Kane, Vernal and Anchor. Digestions involved a 4 hr nylon bag digestion of fresh chopped forage sampled at a vegetative stage in the rumens of fistulated steers. Between 2 and 5 % of the least digested plants were selected in each generation. Cycles 1 and 2 took two years to complete; cycles 3 and 4 took four years each as progeny testing was done. In grazing trials, the cycle-4 population (LIRD-4) had an initial rate of selection approximately 85% of that of unselected Beaver alfalfa and a mean reduced bloat incidence of 62% (1, 2). The goal of the present study was to compare the four parental cultivars (cycle-0) and the four selection cycle populations in a single experiment to determine the response to selection for LIRD and associated changes in forage quality and plant development. Populations were established in 10 replicate nurseries in 1994, with each plot consisting of a 5 m row of 15 plants. Plants were sampled in 1995 and 1996 at a vegetative anda mid-bloom stage. Characteristics measured were intitial rate of digestion (4 h nylon bag digestion), 24 and 72 hour *in vitro* digestions, acid detergent fibre (ADF), neutral detergent fibre (NDF), crude protein (CP), lignin, plant height, stem thickness, leaf percentage and maturity (mean stage by count). Not all characters were measured at both growth stages. Analyses were done over years as there were no year*cycle interactions for any character.

For IRD, there was a sequential decline over cycles for the vegetative sampling, with cycle-4 having an IRD 89% of that of cycle-0. No such sequential decline was found at the mid-bloom stage. Differences in IRD were not significant (p=0.05) due to the high variability of this biological measurement. For other characters, significant differences were found for ADF and NDF for both the vegetative and mid-bloom samples and for leaf percentage and plant height, which were only recorded on mid-bloom stage samples. For NDF and ADF in vegetative stage samples, there were linear increases over cycles, with cycle-4 being 11 gm kg-1 and 8 gm kg -1 higher than cycle 0. This amounted to a 6% increase in both NDF and ADF. For mid-bloom stage samples, increases in ADF and NDF were more curvilinear. The lack of differences in lignin concentration among cycles suggests that increases in fibre concentration have been due to increased cellulose and hemicellulose. This corresponds well to the reported (2) increases in leaf epidermis and mesophyll wall thickness in the cycles of selection for low IRD, and these cells are not highly lignified. No significant differences, nor obvious trends, were found in 24 and 72 h digestions and crude protein concentrations. There was a trend towards increased plant height for all selected cycles and all selected populations had a lower leaf percentage than the source population. There were no differences in stem width or maturity.

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Growth and cultivar effects on alfalfa digestibility

Bernadette Julier, Christian Huyghe and Christian Ecalle
INRA, Station díAmélioration des Plantes Fourragères, F - 86 600 Lusignan, France

We analyzed the effects of cultivar and environment on the decrease of digestibility during growth cycle. Seven cultivars were studied in five locations in France during two regrowth cycles, with four samplings per cycle for four cultivars. Forage yield, whole plant digestibility and leaf/stem ratio were recorded. Stem digestibility was calculated from whole plant digestibility and leaf/stem ratio, assuming a constant leaf digestibility.

All traits were influenced by location, cycle, cultivar and sampling date effects (Julier & Huyghe, 1997). Digestibility and leaf/stem ratio decreased with forage yield, as plants grew. The slope of regression between plant digestibility and yield was the same for all the cultivars but varied with the location. Digestibility decreased quicker in some locations than in others but location effects were not related to average temperatures. At harvest (beginning of flowering), no correlation between yield and digestibility was observed, showing that it is possible to identify high yielding and digestible varieties.

The seven cultivars showed differences in digestibility (Table 1), as a result of various combinations of leaf/stem ratio and stem digestibility. Both high leaf/stem ratio and high stem digestibility are required to achieve a high whole plant digestibility.

Table 1. Adjusted means of plant digestibility, stem digestibility, leaf/stem ratio, and forage yield per cut (g/m_) in analysis of variance, for seven cultivars grown in five locations, and harvested at beginning of flowering in two growing cycles.

	Plant digestibility	***Stem digestibility***	***Leaf/stem ratio***	***Forage yield***
Europe	**68.52**	**54.10**	**0.71**	**586**
Julus	**68.24**	**53.55**	**0.70**	**517**
Lodi	**69.30**	**52.67**	**0.84**	**486**
Luzelle	**68.28**	**52.91**	**0.73**	**536**
Maya	**68.96**	**53.83**	**0.77**	**543**
Natsuwakaba	**67.73**	**50.02**	**0.82**	**468**
63-28P	**70.08**	**54.68**	**0.81**	**522**
M.S. error	**2.71**	**4.97**	**0.015**	**6224**

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Within and between-cultivar genetic variation for alfalfa digestibility

Bernadette Julier, Christian Huyghe and Christian Ecalle
INRA, Station díAmélioration des Plantes Fourragères, F - 86 600 Lusignan, France

The genetic structure (synthetic) of alfalfa varieties and the tetraploidy of the species can generate a large within-variety variation. We compared within-variety and between-variety variation for digestibility and fiber contents on 11 varieties. From 7 to 20 plants per variety were cloned in 15 cuttings, and planted in 1995 in 5-plant-plots in a 3 block design. Space between plants was 10 cm in both directions. Three harvests were made in 1996: 30 May, 04 July, 10 October. Each plot was cut, and forage was dried, weighed, and analyzed for enzymatic digestibility and fiber contents (NDF, ADF, ADL), by prediction with NIRS.

In each cut, analysis of variance was performed with block effect (fixed), variety and plant within variety effects (random). Variety effect represents between-variety variation, and plant within variety effect represents within-variety variation. Variances of random effects (σ _B and σ_W) were calculated. Broad-sense heritability was calculated as (σ _B + σ _W)/(σ _B + σ_W + σ_R).

All effects were highly significant for all traits. Within and between-variety variances are presented in table 1. Large within-variety variances existed for digestibility and fiber contents in each cut, similar or larger than between-variety variances. Within-variety variance for forage yield was much larger than between-variety variance.

Breeding for a higher digestibility in alfalfa should take into account within-variety variation, in addition to between-variety variation. Breeding schemes must be accordingly adapted.

Table 1. Within and between-variety variances and heritabilities for digestibility (Dig), fiber contents (NDF and ADL) and forage yield, in three cuts on alfalfa.

	Cut of 30-05-1996				Cut of 04-07-1996				Cut of 10-10-1996			
	Dig	NDF	ADL	Yield	Dig	NDF	ADL	Yield	Dig	NDF	ADL	Yield
σ_B	0.93	1.30	0.05	2	0.88	0.86	0.04	0.02	1.35	1.59	0.10	2.0
σ_W	1.78	2.55	0.12	291	1.46	2.24	0.07	5.79	1.08	1.39	0.07	67.9
h_	0.50	0.55	0.60	0.74	0.33	0.33	0.27	0.68	0.51	0.50	0.54	0.54

Yield and Stand Reduction Caused by a New Biotype of Blue Alfalfa Aphid in Oklahoma

A.A. Zarrabi, R.C. Berberet, A.D. Bisges, and J.L. Caddel Oklahoma State University, Stillwater, OK 74078, U.S.A.

In 1995, the existence of a new blue alfalfa aphid (BAA) biotype designated as "BAOK90" was confirmed in Oklahoma (Zarrabi et al. 1995). The purpose of research reported here was to describe results of infestation by BAOK90 on yield and stand retention in alfalfa as observed in several types of field experiments. Field experiments were planted in September of 1995 and 1996 with entries having varied levels of resistance to BAA arranged in an RCB design with 6 replications. The experiments were naturally infested by BAOK90 in February /March 1997. Aphid population density and forage yield were estimated in both experiments. In first year stand, peak population densities of BAOK90 ranged from 43/stem in the most resistant entry "54H55" (Pioneer) to 93/stem in "OK08" the susceptible check on 31 March, 1997. Although the yield of 54H55 was significantly higher at first harvest than for two other entries developed in Oklahoma for resistance to BAOK90 ("OK 206" and "OK 207"), seasonal yields were not significantly different. In the second-year stand, peak population densities ranged from 88/stem (OK 207) to 195/stem (OK08) on 26 March, 1997. The entry from Pioneer (54H55) was not included in this experiment. The highest yields at both first harvest and for the season were in OK 206 and OK 207, significantly greater than in the susceptible check (OK08).

Insecticides have been evaluated annually for control of BAA and alfalfa weevil larvae (AW) since 1987. The results for 1987 (the original biotype of BAA was prevalent) and 1997 (the predominant biotype was BAOK90) allow for comparison of the potential for yield reductions. Population densities (ca 4.0/stem) and damage attributable to AW were similar in the 2 years. However, in 1987, the untreated plots yielded nearly 3800 Kg/ha at first harvest with a population of 89 BAA/stem, while, in 1997, BAA numbered 53/stem and damage by the more virulent biotype resulted in virtual destruction of the first crop (167 Kg/ha).

Research was conducted from 1995-97 to evaluate spring-grazing vs. early cutting for insect and weed control, and profitability in forage production. Harvest treatments included cleanly grazing stands with cattle for a 3-4-week period during March; prebud harvest in early April near the time of peak occurrence of BAA and AW; and early bloom harvest in May. On subplots for each harvest option, there were sprayed (insecticide and herbicide as needed) and unsprayed treatments. In 1997, the lowest yields occurred in the unsprayed prebud and unsprayed early bloom harvest treatment combinations. Forage yield was 350 Kg/ha at the first early bloom harvest (unsprayed), primarily as a result of damage by BAOK90, which caused a highly significant loss of stand, as well. Results obtained confirm that since 1991, alfalfa lines selected for resistance to the new BAA biotype BAOK90 tend to have lower aphid populations and produce higher yields in the field evaluations. Evaluations of insecticides and harvest schedules have shown that management programs which do not provide for effective and timely control of BAA result in dramatic reductions in yield and loss of stand in alfalfa.

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Verticillium wilt increases with irrigation in wilt-susceptible alfalfa

Paul G. Jefferson and Bruce D. Gossen
Agriculture and Agri-Food Canada, Swift Current, SK S9H 3X2
Agriculture and Agri-Food Canada, Saskatoon, SK S7N 0X2

Verticillium wilt (VW), caused by *Verticillium albo-atrum*, has occurred in scattered irrigated alfalfa (*Medicago sativa* L.) fields in southwestern Saskatchewan since the late 1970s, but does not cause detectable injury in dryland fields. We were interested to examine the impact of irrigation on the rate of spread of VW in this semiarid region. A line-source irrigation system was used to produce an irrigation gradient on two adapted cultivars, Beaver, a VW-susceptible cultivar commonly used for irrigated production in the region, and Barrier (VW-resistant). Plots were seeded at Swift Current, SK in 1994, in a commercial field where VW was observed in the previous year. The irrigation gradient was sub-divided into six water level treatments within each cultivar plot and each treatment combination was replicated four times. Irrigation plus precipitation from April to August each year averaged 27, 29, 31, 33, 35 and 35 cm for gradient treatments one to six, respectively, over 1995 to 1997. Stand density and counts of VW-infected plants per subplot were recorded in spring, and forage yield was assessed twice per season. In both 1995 and 1996, Beaver had more VW-infected plants than Barrier, and the number of infected plants increased with increasing irrigation water. As expected, the number of VW-infected plants in Barrier showed no response to irrigation. However, water restrictions at this site in 1996 and 1997 resulted in delayed and relatively low amounts of irrigation. This resulted in a steady decline in the number of infected plants in the trial from 1995 to 1997. Severe stand thinning associated with winter injury was observed in spring of 1997. Stand density in Beaver was negatively correlated to VW-infected plant counts from 1995 and 1996. Barrier, however, exhibited no relationship between these variables. Forage yield increased with increased irrigation in each year, except for Beaver in 1997. Forage yield of Beaver did not respond to irrigation in 1997 because stand density had declined to 60% at water level six compared to 78% at water level one. The stand density of Beaver at water level one was similar to that of Barrier at all water levels. We concluded that impact of VW on irrigated stands was correlated to the level of irrigation water applied, and that VW does not affect susceptible alfalfa cultivars on dryland because its rate of spread from plant to plant is very low in most years.

Swathing with Early Raking for Control of Alfalfa Weevil

Sue L. Blodgett, Andrew W. Lenssen, and S. Dennis Cash
Departments of Entomology and Plant, Soil, and Environmental Sciences, Montana State University, Bozeman, MT 59717

The alfalfa weevil, *Hypera postica* Gyllenhal, is the most important phytophagous pest of alfalfa in the western USA. Several cultural, nonchemical methods to manage alfalfa weevil larval populations have been tested, but only early cutting has shown a moderate level of success. When weevil numbers are high, early harvest may not prevent damage to regrowth, necessitating stubble treatment with an insecticide after the forage crop is removed from the field to allow normal regrowth. Field trials were conducted in 1996 and 1997 to determine if early harvest combined with early raking would provide more reliable control of alfalfa weevil larval populations than early harvest alone. Preharvest mean $\pm$ SEM alfalfa weevil larval numbers were 223 $\pm$ 17 per 0.1 m^2 for the early harvest plus early raked forage and 188 $\pm$ 69 per 0.1 m^2 for early harvest alone. Post-baling mean $\pm$ SEM larval numbers in stubble were 3.8 $\pm$ 1.3 per 0.1 m^2 for the early harvest plus early raked forage and 6.2 $\pm$ 2.0 per 0.1 m^2 for the early harvest alone. Forage quality was not influenced by early raking. Concentrations of crude protein, and neutral and acid detergent fibers did not differ between early harvested early raked and early harvested forages. Early cutting followed by early raking may be an effective management tool for decreasing damage by alfalfa weevil larvae and reducing pesticide use without compromising yield or nutritive value of forage. A field study was conducted in 1996 and 1997 to determine larval survival and locations following alfalfa harvest as dry matter concentration changed in forage swathed by self-propelled mower conditioners. Live alfalfa weevil larvae were quantified in, under, and between windrows. Following cutting, the percentage of live weevil larvae residing in swathed forage decreased quadratically as swath dry matter percentage increased (y = 43.0 + 1.66x - 0.022x2, R2 = 0.66). Conversely, larval populations in stubble between windrows increased with increasing swath dry matter (y = 36.4 - 1.29x + 0.016x2, R2 = 0.67). Larval populations in stubble under windrowed forage increased slightly as windrowed forage dry matter increased (y = 22.2 - 0.46x + 0.006x2, R2 = 0.25). Applications of these results for the development of alfalfa forage harvest systems for improved cultural control of alfalfa weevil will be presented.

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A Potential Source of Resistance to Summer Black Stem.

K.D. Glover, P.C. St. Amand, D.Z. Skinner, and D.L. Stuteville.
Kansas State University and USDA-ARS; Manhattan, KS

Summer Black Stem, incited by *Cercospora medicaginis* (Ellis and Everh.), is a common and chronic alfalfa production constraint. Chocolate-brown leaf spots with irregular margins are the most obvious symptoms (Fig. 1). Relatively low levels of resistance to this disease are present in many alfalfa varieties.

Figure 1. Leaf spots from artificial inoculation of *Cercospora medicaginis* on tetraploid alfalfa.

The objectives of this research were to: 1) evaluate the performance of KS211 using a growth chamber screening procedure, 2) determine whether number of inoculations significantly altered test results, 3) determine consistency of screening results among tests, and 4) derive a broad-sense heritability estimate for Summer Black Stem resistance using tetraploid varieties ranging from resistant to susceptible. Twelve entries were included in a split-plot randomized complete block design with inoculations as main-plots and cultivars as sub-plots. Each test contained four blocks and five tests were performed. KS211 was found to be significantly more resistant than all but three entries (P = 0.01). Four inoculations did not present significantly higher disease pressure than one inoculation. Therefore, all main plots were considered equal in subsequent analyses and the number of blocks within a test increased from four to eight. Pearson product correlations and Spearman rank correlations among tests were very high (> 0.90) when testing cultivars with significantly different resistance levels. Correlations among tests were low to moderate (< 0.50) when tests included a large number of cultivars where resistance levels were not significantly different. A broad-sense heritability estimate of 0.747 was calculated using 12 cultivars as the reference population. The moderate broad sense heritability estimate **s**uggests that increasing levels of resistance to Summer Black Stem should be possible. Results indicate that KS211 is a good source of resistance to Summer Black Stem.

Studies on the Biology of Brown Root Rot (*Phoma sclerotioides*) of Alfalfa in Wyoming

Charla R. Hollingsworth[1], Fred A. Gray[2], Tom E. Heald[3] & Dave W. Koch[4]
[1,2,4]Department of Plant Sciences, and [3]Cooperative Extension Service University of Wyoming, Laramie, WY 82071

Brown root rot (BRR) of alfalfa caused by the soil-borne fungus *Plenodomus sclerotioides* (now *Phoma sclerotioides*) was first reported from Alberta and Saskatchewan, Canada (Sanford, 1933). Symptoms consist of brown, slightly sunken, necrotic lesions, which appear on the tap or lateral roots and rootlets of mature plants (Davidson, 1990). Black pycnidia (asexual fruiting bodies) are frequently present on or in necrotic root tissue. Plants with severe root rot may winter-kill or have poor spring growth. If secondary lateral roots are produced, diseased plants may partially recover. Fields with BRR may show severe winter-kill during certain years. BRR was tentatively identified in Wyoming from 3-year old alfalfa stands showing severe winter-kill in the spring of 1996 (Gray, et al., 1997). This disease had not previously been reported from the USA. BRR has since been identified in over 2,500 acres of irrigated alfalfa in southwestern Wyoming. Isolation of diseased plant roots resulted in a pycnidial fungus which was later identified as *P. sclerotioides* when grown on potato dextrose agar at 10oC (50oF). In vitro growth of *P. sclerotioides* occurs best on dilute V-8 juice agar. Pycnidia and pycnidiospore production, necessary for positive identification of the fungus, require a 6-8 week growth period at 10oC (50oF). *Phoma sclerotioides* can be differentiated from *P. medicaginis* var. *medicaginis* and *Stagonospora meliloti*, both pycnidia producing fungal pathogens of alfalfa, by cultural and morphological characteristics. Inoculum, produced on sterile barley, remained viable for 10 months at -14^{o}C (6^{o}F) and for only 5 months at 24^{o}C (75^{o}F). In January of 1998, five-month-old alfalfa plants (cultivar 'Multiplier'), were inoculated and placed outside for an abbreviated winter season. A high frequency of root infection by *P. sclerotioides* was observed the following spring. Recent observations of a 7-year-old alfalfa cultivar trial located near Eden, Wyoming show a marked difference in alfalfa persistence in the presence of BRR. This suggests field resistance may be present in some USA-developed alfalfa cultivars.

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Alfalfa diseases in north-central Mexico

Y.I. Chew M., J.J. Márquez O., and J. Santamaría C.
Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias. C.E. La Laguna. Apdo. Postal 247, CP 27000 Torreón, Coah. Mexico.

Alfalfa is the most important forage crop (35,000 ha) at La Laguna, the largest dairy production area in north-central Mexico. However, despite that some plant diseases are a major cause for low alfalfa production in certain areas of La Laguna, there is a lack of general information of how plant diseases affect alfalfa production in this region. Therefore, our objective was to identify the most important diseases, to determine their time of appearance throughout the year, and their impact on forage production in the area.

We sampled 20 farms of La Laguna in March, June, August, and November of 1995 and 1996. Thirty whole plants were dug from the field at each farm. In March and November of both years we took soil samples (0-50 cm depth) to determine nematode incidence. In March and November of 1997 samplings were made at six farms to estimate yield losses caused by crown rot. Plants from each ranch were classified based on degree of damage (severity) caused by the crown rot complex (0=no damage; 1=1 to 25% damage; 2=26 to 50% damage, and 3=>50%). We measured fresh and dry weight of twenty-five plants from each rank of the scale. Forage losses at one, two, and three years of stand age were estimated using yield:severity ratios and percentages of damaged plants.

The most important diseases found in the area were crown rot (*Fusarium* spp, *Rhizoctonia solani*, *Phoma* sp, and *Colletotrichum* sp), anthracnose (*Colletotrichum* sp), Texas root rot (*Phymatotrichum omnivorum*), mildew (*Peronospora trifoliorum*), and rust (*Uromyces striatus*).

Crown rot incidence was almost 100% for both years, starting in the summer. Anthracnose was observed in wilted stems, but also associated to crown rot (85% in 1995 and 70% in 1996). Stand age was positively correlated to damage caused by crown rot and anthracnose. Texas root rot had an incidence of 55% in 1996 and 50% in 1996, starting in May, when temperatures increased.

Mildew was found in March 1995 (50%) and March and November 1996 (20%). Rust showed up in August 1995 (30%) and August and November 1996 (20%). Differences among years were caused by increased moisture conditions in October 1996.

Nematodes found were *Pratylenchus* sp, *Helicotylenchus* sp, *Tylenchorhynchus* sp, and *Xiphinema* sp, but they were not considered a problem.

We estimated that alfalfa crown rot reduces dry matter forage yield 11.6, 28.0, and 33.33% in the first, second, and third year of stand age, respectively.

Trichome Density Influences Drying Rate of Alfalfa Forage.

A.W. Lenssen, J.D. Banfield, and S.D. Cash
Departments of Entomology, Mathematics and Statistics, and Plant, Soil, and Environmental Sciences, Montana State University, Bozeman, MT 59717

Alfalfa (*Medicago sativa* L.) cultivars with erect glandular hairs have been developed and released for potato leafhopper [*Empoasca fabae* (Harris)] resistance. Trichomes decrease water loss in some plant species, but their influence on drying rate of harvested alfalfa forage is unknown. We conducted a study measuring water loss from field-grown, harvested forage of three erect, glandular-haired alfalfa germplasms, KS161, KS210, and KS224, eglandular 'Arc', and 'Kenland' red clover (*Trifolium pratense* L.). Harvests were taken once in 1995 and 1997, and four times in 1996. Plant height, phenological stage, upper and lower stem diameters, leaf:stem ratio, and apical stem densities of simple, procumbent glandular, and erect glandular trichomes were determined. Forage drying rate constants were calculated by a combination of parametric and nonparametric modeling techniques. The harvest x germplasm interaction was significant for all parameters measured. The density of erect glandular hairs on stems differed among entries in all 6 harvests, with mean values being similar to those reported by Danielson et al. (1989) for the three perennial *Medicago* species, *prostrata, glandulosa, and glutinosa,* that provided the original sources used to transfer the erect glandular hair trait into alfalfa. Despite populational differences in erect glandular hair densities, drying rate constants only differed among alfalfa entries in two of the six trials. Correlations between density of total trichomes (simple + procumbent glandular + erect glandular) and drying rate constants were negative and significant, indicating that increasing total trichome density may retard water loss from cut forage. Correlations of erect glandular hair density with drying rate constants were generally low and nonsignificant, except for one harvest. Erect glandular hairs, at the densities measured in this study, generally did not influence drying rates of alfalfa forage. However, density of total trichomes was significantly and negatively correlated with drying rate for each of the five entries, with correlation coefficients ranging from -0.45 to -0.67. Our results indicate that selection for decreased pubescence may increase water loss from harvested forage. Further research is necessary to determine if drying rate of alfalfa forage can be improved through selection for reduced density of trichomes.

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Differentiating *M. sativa* ssp. *sativa* and ssp. *falcata* accessions using molecular markers

Patricia M. Cazcarro and E. Charles Brummer
Iowa State University, Ames, IA 50011

Cultivated alfalfa, *Medicago sativa* ssp. *sativa*, grown in most regions of the world owes few of its agronomic qualities to ssp. *falcata*. However, ssp. *falcata* possesses desirable cold, drought, and grazing tolerances (Oakley and Garver, 1917). Further, significant heterosis has been expressed in some sativa x falcata crosses (Wilsie, 1958; Sriwatanapongse and Wilsie, 1968). Several studies have shown falcata germplasm to be genetically distinct from sativa (Kidwell et al., 1994; Crochemore et al., 1996; Ghérardi et al., 1998). The objective of this experiment is to identify falcata accessions most distinct from sativa germplasm to guide development of improved falcata populations with complementary allelic structures to Midwestern U.S. breeding populations. We are examining putatively native accessions to study the geographical distribution of alfalfa diversity to guide germplasm enhancement efforts. The native ranges of falcata and sativa overlap to a considerable extent, but allopatric regions also occur: solely falcata grows in northern regions of Russia, Scandinavia, Mongolia, and China while sativa is the only subspecies in southern Europe, the Middle East, and Northern Africa (Ivanov, 1980; Lesins and Lesins, 1979). Ten accessions of each subspecies were selected from regions of coexistence and ten sativa and nine falcata accessions were selected from allopatric habitats, forming four "superpopulations" of sympatric sativa, sympatric falcata, allopatric sativa, and allopatric falcata. In addition to these 39, two *M. prostrata* accessions are included as an outgroup. Several types of molecular markers (RAPD, SSR, and RFLP) are being screened against two individuals of each accession. Genetic distances, calculated from the marker data, are used to cluster the various accessions. We are less interested in within population variation for this experiment, but rather in how the variation is structured over large geographical zones. Several questions will be discussed: Do sympatric and allopatric accessions form distinct clusters within each subspecies? Do sympatric accessions of each subspecies resemble each other more than they resemble their respective allopatric accessions? Though alfalfa populations contain considerable genetic diversity, significant differentiation among populations has also been reported (e.g Brummer et at., 1991). To get a clear picture of allelic differentiation among these accessions, a large number of polymorphic bands need to be screened. Results will be discussed relative to the usefulness of this method for detecting potentially useful germplasm.

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Blossom Blight Reaction of Alfalfa Cultivars in Growth Cabinet Trials

Zhiwen Lan and Bruce D. Gossen
Saskatoon Research Centre, Agriculture and Agri-Food Canada, Saskatoon, SK S7N 0X2

Alfalfa seed is an important crop in many areas of western Canada. Blossom blight, caused by *Botrytis cinerea* and *Sclerotinia sclerotiorum*, is a serious constraint to production when cool, wet weather occurs during flowering. The symptoms caused by *B. cinerea* begin as small, water-soaked spots on blossoms. The spots coalesce rapidly, affecting the whole flower, and mycelium often grows from flower to flower, quickly attacking all of the flowers in a raceme. Infected blossoms abscise prematurely without setting seed. This disease has only recently been recognized in western Canada, so we were interested to determine if locally-adapted cultivars differed in disease reaction. The blight reaction of 12 alfalfa cultivars, representing a range of winter hardiness and plant architecture types, was evaluated in inoculated detached flower trials and growth cabinet tests in 1997-98. There were consistent differences among cultivars in degree of susceptibility; cvs DK 135, OAC Minto, and Iroquois were significantly less susceptible than cvs Algonquin, Apollo II and Heinrich. For example, in whole plant inoculation tests, the incidence of flower infection after 48 hours of incubation, of Iroquois, OAC Minto and DK 135 was

36%, 36% and 40%, respectively, and 75%, 79% and 71% for Algonquin, Apollo II and Heinrich. Similar differences were observed after 12 and 24 hours of incubation in tests using detached flowers under controlled conditions. However, all of the cultivars in the trial were at least moderately susceptible to infection. Parallel studies are being conducted by Dr. Holley at Brooks AB. The impact of flower orientation and color on infection incidence was also assessed. Flowers of cv Vernal that faced upward had a much lower incidence of infection (16% infected) than downward facing flowers (86%). Purple flowers of cv Iroquois had a lower incidence of infection than white flowers (61% vs. 81%) after 24 hr incubation in a detached flower study, but there were no differences associated with flower color in Apollo II (purple vs. white flowers) or AC Nordica (purple vs. yellow). Trials are underway to assess cultivar reaction in field trials at Saskatoon SK and Brooks AB. It may be possible for growers in regions where blossom blight frequently occurs (e.g. the Peace River region of northern Alberta) to reduce the risk of serious yield loss by selecting cultivars that are less susceptible to blossom blight.

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Performance of Two Pea Aphid Biotypes on Glandular Haired Alfalfa

T.J. Davis, D.B. Hogg and A.M.A. Mohamed
Department of Entomology, University of Wisconsin, Madison, WI 53706

Pea aphid, *Acyrthosiphon pisum* (Harris), is an occasional pest of alfalfa in the midwestern U.S. Pea aphid populations in Wisconsin consist of what are believed to be two biotypes that differ in color, hereafter referred to as the green form and the pink form. The two biotypes occur together and appear to coexist in alfalfa fields, although the green form tends to maintain a higher density of the two. In 1997, glandular haired alfalfa varieties with professed resistance to potato leafhopper were released commercially. The purpose of this study was to investigate and compare the performance of the two pea aphid biotypes on glandular haired and non-hairy alfalfa.

In a no-choice study, life history attributes of the two biotypes were compared on the glandular haired cultivar 'Trailblazer' and the non-hairy cultivar 'Rushmore'. The study was conducted under fluctuating temperatures in a greenhouse with 16L:8D photoperiod and approximately 70% RH. Daily high and low temperatures during the study averaged about 22%C and 14%C, respectively. Aphids were reared individually in dialysis tube cages on alfalfa stems and were checked daily for development (molting), mortality and reproduction (nymphs).

Aphid developmental period (days to adulthood and first reproduction), reproductive period, and daily and total reproduction were analyzed using ANOVA with main factors aphid color form and alfalfa cultivar. No statistical differences were detected in developmental or reproductive periods. However, there were significant differences for color form in daily reproduction (green > pink) and for cultivar in total reproduction (Rushmore > Trailblazer). Also, survivorship values of the green aphids after 40 days on Trailblazer and Rushmore were 40% and 90%, respectively, whereas survivorship values for the pink aphids were 60% and 100%, respectively.

In addition, age-specific schedules of survivorship and reproduction were used to estimate life table parameters, most prominently the intrinsic rate of increase which provides an index of potential population growth, for the four combinations of color form and cultivar,. Despite the influence of cultivar on both total aphid reproduction and survivorship, the effect of cultivar on intrinsic rate of increase was not statistically significant. This unexpected outcome appears to have happened because 1) peak aphid reproduction occurred earlier on Trailblazer than on Rushmore, thus contributing more to potential population growth rate, and 2) aphid mortality on Trailblazer occurred after peak reproduction had already taken place. In contrast, the effect of aphid color form on intrinsic rate of increase was statistically significant, with the green form having a slightly higher rate than the pink form.

These results suggest that the glandular hair trait involved with alfalfa resistance to potato leafhopper has no measurable effect on pea aphid population growth. In addition, the difference in population growth rates between the green and pink color forms may contribute to the higher densities of green pea aphids observed in field populations and provides support for biotype designation.

Developing an Alfalfa CD-ROM and WWW Information System

David B. Hannaway, Dept. of Crop & Soil Science, Oregon State Univ., Corvallis, OR 97331-3002; Dan Undersander, Department of Agronomy, Univ. of Wisconsin, Madison, WI 53706; Dan Putnam, Department of Agronomy & Range Science, Univ. of California, Davis, CA 95616; Garry Lacefield, West Kentucky Research & Education Center, Univ. of Kentucky, Princeton, KY 42445; Neal P. Martin, Department of Agronomy & Plant Genetics, Univ. of Minnesota, St. Paul, MN 55108; John Caddel, Department of Agronomy, Oklahoma State University, Stillwater, OK 74078

This project will develop an educational CD-ROM and WWW segment for alfalfa production, management, marketing, and utilization. These products will be useful for all individuals involved in marketing, advising farmers and ranchers, and producing or using alfalfa. University alfalfa experts from across the US will develop materials in concert with industry experts and experienced alfalfa producers. Experts will be organized into regional teams to assure that geographical, climate, and soil "agroecozone" differences are included in management recommendations. This will result in a comprehensive multimedia information resource (including text, graphics, full color pictures, video and audio segments, and instructor materials) for technically correct and up-to-date information readily available and easy-to-use. Topics will include variety selection; establishment (no-till and traditional techniques); growth and development; fertility, harvest, grazing, irrigation, and pest (weed, insect, disease, nematode, vertebrate) management; hay quality and testing; silage making; utilization by dairy, beef, sheep, horses, and other livestock and wildlife; marketing and production statistics, university and industry contacts, references, and alfalfa-related organizations. The CD will contain active links to the WWW for rapidly changing information. Yearly updates will be available at a reduced cost following initial purchase. Development will require a combination of alfalfa-related agribusiness sponsorship and USDA grant funding. A prototype is available for inspection at the following URL:**http://www.forages.css.orst.edu/Topics/Species/Legumes/Alfalfa/AISDS/**

Growth and Yield of Introduced Alfalfa (*Medicago sativa* L.) Cultivars in Korea

Moo-Sung Kim and Se-Young Kim
Department of Agronomy, Kyung-Hee University, Suwon Campus, Suwon, Korea

This study was conducted to select the best adapted alfalfa cultivars among 32 introduced cultivars from the United States into Korean environmental conditions. Plant height and dry matter yield per cultivar was determined. Three replications of 32 plots incomplete randomized block were used in the experimental field at Kyung-Hee University, Suwon Campus, Korea to evaluate. Alfalfa cultivars used in this experiment included dormant types, Husky, Milkmaker, Pioneer brand 526, Pioneer brand 5444, Pioneer brand 532, Pioneer brand 545, Drummor, Blazer, DeKalb brand 120, DeKalb brand 135, Raidor, Challenger, Arrow, semi-dormant types, LS-1920, LS-214, Pioneer brand 555, Pioneer brand 581, Amador, Pike, Salute, Magnum, DeKalb brand 167, DeKalb brand 187, Decathlon, Armor, Diamond and non-dormant types, Pioneer brand 5929, Florida 77, Maxidor, Pierce, and Sapphire. Test plots were seeded conventionally prepared seed beds. Lime and fertilizers were incorporated into the soil prior to seeding. Seeds were inoculated with 3 strains of *Rhizobium* bacteria. Plots were seeded in 3 rows with 20cm row spacings. Plots were harvested 4 times by cutting in the one-tenth blooming stage during the growing season. Plant height and dry matter yield were measured. Mean values of 4 harvests in a year for plant height in centimeters and dry matter yield in tons per acre were recorded. The seven highest ranked cultivars from the 32 alfalfa cultivars tested for plant height are: Diamond>Milkmaker>Arrow>Challenger>Sapphire>DeKalb brand 135>LS-1920, and the seven lowest ranked cultivars are: Pioneer brand 5929<Maxidor<DeKalb brand 167<Pierce<Blazer<Raidor<Pioneer brand 532. The seven height ranked cultivars for dry matter yield are: PikeLS-1920>Challenger>Salute> Diamond>Decathlon>Pioneer brand 581 and the seven lowest ranked cultivars are: Maxidor< Pioneer brand 5929< Pioneer brand 532<Florida 77<Pierce<Magnum<Blazer. Dry matter yield was generally proportional to plant height. The leading cultivars for dry matter yield under Korean environmental conditions were: Pike, LS-1920, Challenger, Salute, and Diamond among the 32 alfalfa cultivars tested. All of these cultivars are semi-dormant types except Challenger all of which can be grown under moderate temperatures.

Phytochemical Characteristics of Alfalfas Grown from Germplasm Obtained from the USDA Collection

Pete Debus, Pat DiPaola, Rick Leitz
Rehnborg Center for Nutrition and Wellness, Nutrilite Division of Amway

The majority of research directed toward the phytochemical characterization of alfalfa has been approached from the traditional use of alfalfa as an animal feed. Relatively little work has been done to assay for the phytochemical profile of alfalfas in terms of human nutrition. We have investigated the content of three classes of phytochemicals: carotenoids, tocopherols and saponins. Carotenoid and tocopherol levels were measured using HPLC. Saponins were estimated using a bovine serum hemolysis technique. Additionally, these alfalfas were assayed to determine their ability to function as an antioxidant using a test tube assay developed to measure the antioxidant activity of aqueous extracts of fresh plants. These assays were performed on 47 varieties of alfalfa obtained from the USDA collection representing various regions from around the world. Statistical analysis has shown a correlation between certain phytochemical constituents and the latitude from which the germplasm originated.

Fertilizer Response of Irrigated Alfalfa in Southern Alberta

R.C. McKenzie[1], S.A. Woods[1], R.H. McKenzie[2] and L. Kryzanowski[3]
[1]Soil & Water Agronomy Unit, Crop Diversification Center - South,
Alberta Agriculture Food and Rural Development (AAFRD), Brooks,
AB, Canada, [2]AAERD, Lethbridge, AB, [3]AAFRD, Edmonton, AB

Alfalfa is an important crop in the irrigated areas of Southern Alberta, however, it frequently receives lower inputs of fertilizers than other crops. A survey of 99 irrigated alfalfa fields was conducted in southern Alberta. Soil phosphorus (P) was found to be low in 70% of the alfalfa fields and marginal on 11% of the fields, but plant tissue P was found to be deficient in only 43% of the fields. Soil potassium (K) was found to be marginal on 12% and adequate on 88% of the alfalfa fields but tissue K was deficient on 79% of the fields. Soil nitrogen (N) was low on 96% of the fields sampled and marginal on 2% of the fields yet tissue N was low on only 1% of the fields. To address the questions that arose from the conflicting results of this survey, a field experiment was conducted to measure the increases in forage yield to various rates and methods of application of P, K and N fertilizers. Phosphorus responses could be best predicted by a combination of soil and tissue tests. On high pH soils, the Kelowna method of determination of soil P was more reliable than the Miller-Axley method in producing a P deficiency. Shallow banded P was equally effective as broadcast P. Annual applications of P over 3 years gave similar yields to one batch application. Soils with marginal or adequate levels of K produced alfalfa with deficient levels of tissue K. These same soils did not give a significant increase in forage yield with K fertilizers. Nitrogen fertilizers increased the forage yield in the first harvest after application. It was not measured how much of this increase was due to increased growth of weeds. Tissue nutrient levels in Alberta fields differed considerably from sufficiency levels developed in the USA. This suggests a need for refining these standards for the local conditions, which include high pH soils, short growing season and cool night temperatures.

Response to selection in the development of a bloat-tolerant alfalfa in Argentina

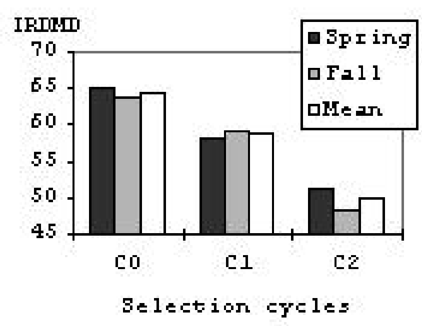
D. H. Basigalup, C. V. Castell, V. Arolfo and M. L. Benitez
EEA Manfredi, Cordoba, Argentina
Partially financed by PRODUSEM, Pergamino, Argentina

Bloat is a very serious problem for Argentine beef and dairy production. In 1991, INTA initiated a breeding program to select nondormant, bloat-tolerant alfalfa cultivars through cycles of phenotypic recurrent selection using the modified nylon bag technique. The initial results of this program, including the production of Cycle 1 seed (C-I) were published in a previous report (1).

During 1995/96, 1,195 C-I plants were evaluated for *in situ* initial rate of dry matter disappearance (IRDMD) after 4 h in the rumen of fistulated steers. While the average IRDMD for the spring (November) evaluation was 58.05%, the mean value for the fall (April) evaluation was 59.30%. The general average for the C-I plants was 58.67%. Ninety-eight genotypes that consistently exhibited lower IRDMD values were cloned and intercrossed in the greenhouse during July-August 1996.

Harvested seed conformed the Cycle 2 (C-II) seed.

In March 1997, 1,300 C-II plants were transplanted to the field to be evaluated twice for their IRDMD during the 1997/98 season. The spring (October) and fall (March) evaluations had mean values for IRDMD of 51.39% and 48.22%, respectively. General average for the C-II plants was 49.81%. In May 1998, 148 C-II plants that consistently exhibited lower IRDMD values were selected and cloned in the greenhouse. They will be interpollinated during July-August 1998 in order to produce Cycle 3 (C-III) seed.



Based of general averages, there has been a 14.61% reduction in the IRDMD between C-0 and C-II populations (Figure 1). We conclude that selection has been effective in reducing alfalfa *in situ* IRDMD.

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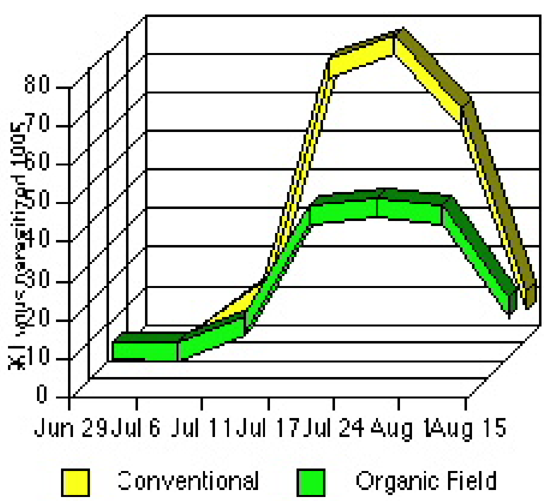
Figure 1. Response to selection for lower IRDMD

Parasitism of *Lygus* spp. in Conventional and Organically Managed Alfalfa Seed Fields.

J. Soroka, W. May[1], H. Loeppky, and D. Murrell[2]
AAFC, Saskatoon Research Centre, Saskatoon, SK, S7N 0X2,
[1]AAFC Indian Head Research Farm, Indian Head, SK, S0G 2K0, [2]Newfield Seeds, Nipawin, SK, S0E 1E0



In western Canada, lygus bugs (*Lygus* spp., Heteroptera: Miridae) are parasitized by a native Braconid wasp, *Peristenus pallipes* Loan. To determine the phenology of parasitism of lygus bugs in alfalfa seed fields and to discern the effects of insecticide application on lygus and parasitoid populations, we monitored lygus populations in a conventionally managed and an organically managed seed field for three years. Research was conducted in a seven year old field of alfalfa cv. Rangelander, which was sprayed with an insecticide for lygus control shortly before leafcutting bees were placed in the field each spring, and a three year old field of alfalfa cv. Peace managed organically near Shellbrook, Saskatchewan (53°13'N Lat., 106°24'W Long.). Insect populations were monitored weekly from the middle of June until the end of August by taking five 180° walking sweeps in each of four sections of the respective fields with a standard 38 cm insect net. Lygus bugs were categorized into three groups: first, second, and third instar nymphs, fourth and fifth instar nymphs, and adults. Collected lygus nymphs were dissected shortly after capture and the levels of parasitism per age group were recorded. The numbers and percentages of parasitized lygus were compared using the T-test procedure of SAS (SAS 1989). In all three years in the conventionally managed seed field, which was sprayed with insecticide for plant bug control prior to leafcutting bee release, parasitoid population development was synchronous with that of its lygus host. Patterns of population growth of both species developed similarly and peaked at the same sampling date. Parasitism in the organically managed field, however, was not as closely aligned with lygus population growth. Similar population patterns were found between the two insect species in 1994, but lygus and wasp numbers were very low in that year. In 1995, levels of parasitism of small nymphs parasitism development in both nymphal categories was later than unparasitized lygus population development by about one week. On 22 of 25 sampling dates over all three years, absolute numbers of parasitized lygus were greater in the organic than in the conventionally managed field, with the differences being significantly higher on 10 of those dates. However, because of the associated larger numbers of unparasitized lygus in the organic field, on 19 of 25 dates the proportion of parasitised lygus was greater in the conventional than in the organic field (Figure), significantly so on nine of those dates.

Reference

SAS Inc. 1989. SAS/Stat User Guide Version 6.06. SAS Inst., Cary, NC. lagged behind lygus population growth, and in 1996.

Fall harvesting management affects the accumulation of specific C and N reserves in alfalfa

C. Dhont[1*], P. Nadeau[2], Y. Castonguay[2], G. Bélanger[2] and F.-P. Chalifour[1]
[1]Université Laval, Ste-Foy, QC, Canada, G1K 7P4 and [2]Agriculture and Agri-Food Canada, Ste-Foy, QC, Canada, G1V 2J3

Fall cutting management is a determinant factor of alfalfa persistence in northern climates. In eastern Canada, the existing recommendation is to not harvest alfalfa during a critical rest period of 4 to 6 weeks from early September to mid October. The negative effect of a fall harvest on alfalfa persistence and the following spring regrowth has been historically attributed to a reduction in the levels of organic reserves, especially non-structural carbohydrates (TNC). Although high TNC accumulation used to be associated with a superior persistence and a more vigorous regrowth, recent reports pointed out the role of specific carbon (C) and nitrogen (N) reserve components in winterhardiness and in the vigor of spring regrowth. Soluble sugars such as sucrose, raffinose and stachyose were found to be closely related to the acquisition of cold tolerance and are thought to have important cryoprotective functions. On the other hand, specific proteins and amino acids were shown to constitute N pools that markedly influence shoot regrowth and tolerance to defoliation.

This study was undertaken to assess the impact of contrasting fall harvesting treatments (with or without fall harvest) on regrowth in relation to the quantitative and qualitative evolutions in C and N reserves in alfalfa taproots during fall and winter. The experiment was conducted under simulated winter conditions in an unheated greenhouse, with two alfalfa cultivars(AC Caribou and WL 225) differing in winter adaptation. Plants were sampled in November, January and March and assessed for their regrowth potential and C and N reserves. Fall harvest markedly reduced shoot regrowth in both cultivars. Cryoprotective sugars (sucrose, raffinose and stachyose) accumulated to higher levels in the hardier cultivar (AC Caribou). The two harvest treatments did not affect the accumulation of TNC. However, there was a tendency for superior accumulation of cryoprotective sugars in plants harvested in fall. The fall harvest also significantly reduced amounts of total N and amino acids such as arginine and histidine. There were only minor differences in concentrations of total soluble proteins between harvest treaments. In both cultivars, we noted a marked accumulation of some proteins during fall and winter, especially a 19 kD MW. This accumulation however is affected by the cultivars and harvest treatments.

Our results confirm the importance of N reserves for spring regrowth of alfalfa. This information will help to optimize fall harvest management of alfalfa and to develop new selection approaches to improve its persitence in northern climates.

Alfalfa Symbiotic Dinitrogen Fixation in the Argentine Pampean Region

R. Racca (Coordinator), D. Basigalup, E. Brenzoni, O. Bruno, C. Castell, D. Collino, J. Dardanelli, M. Diaz Zorita, J. Duhalde, N. Gonzalez, W. Hansen, N. Heinz, F. Laich, A. Lopez, O. Peralta, A. Perticari, A. Quadrelli, E. Rivero, N. Romero and R. Sereno.

PRONALFA National Project - INTA
IFFIVE - Cno. 60 Cuadras, KM $5^{1/2}$ (5119) Cordoba, Argentina

In 1993/97, INTA conducted a multi-site research project (PRONALFA) to quantify alfalfa biological dinitrogen fixation (BDF) and to identify factors that could affect the efficiency of the alfalfa-rhizobia symbiosis. Five identical field experiments, using cultivars "Monarca ISP" (FD=8) and "Victoria ISP" (FD=6), were planted at Rafaela, Manfredi, Gral. Villegas, Anguil and Barrow Experiment Stations (Pampean Region). Forage yield, proportion of N derived from BDF, evolution of physical and chemical soil parameters, nodule dynamics, soil profile rhizobia distribution, and crop water balance were determined in each location.

Average forage yields (dryland conditions) ranged from 4.97 T DM ha^{-1} yr^{-1} at Barrow (4-5 cuts $season^{-1}$) to 19.32 T DM ha^{-1} yr^{-1} at Rafaela (6-7 cuts $season^{-1}$). Using the ^{15}N dilution technique and non-N_2-fixing control plants, it was determined that means of 129.9 (Barrow) to 410.1 (Rafaela) Kg of N ha^{-1} yr^{-1} derived from BDF were removed only in alfalfa forage. About

50% to 72% of total herbage N needs were derived from BDF. Including the build-up of symbiotically fixed N in soil organic matter, it can be estimated that at least a total of approximately 200 to 650 Kg of N ha^{-1} yr^{-1} were fixed by crop.

Both native and inoculated rhizobia were found along the soil profile (0-170 cm). At the deepest layers, active, laminate-shaped nodules were found in cracks and inter-aggregate spaces. Subsoil (0-30 cm) nodule numbers and rhizobia populations were highly fluctuating throughout the years and highly correlated with soil water content. At deeper layers (70-170 cm), nodule numbers were more stable and less affected by weather conditions. Whenever possible for the plant to form new fibrous roots there was *de novo* nodule formation, indicating the presence of active rhizobia along the whole profile. Foliar Water Potential (Q w) of -1.8 MPa and a Relative Water Content of 70% stopped BDF. Soil organic matter content &πουνδ; 5% did not prevent nodulation but affected its expression.

EFFECT OF DIFFERENT GRAZING PERIODS ON YIELD AND PERSISTENCE OF ALFALFA CULTIVARS FROM DIFFERENT FALL DORMANCY GROUPS

R. Rossanigo
EEA Marcos Juarez-INTA, Argentina

The ideal management for alfalfa must combine high dry matter production with high quality and good persistence. Several studies have demonstrated that this could be feasible under grazing conditions. However, the negative effect of continuos grazing over vigor and persistence of alfalfa plants is well documented. On the other hand, the effect of appropriate rotational grazing systems in increasing forage yield, beef production ha^{-1}, and persistence, and in decreasing weed invasion, is also well known. The specific needs for dairy or beef operations may introduce changes in the appropriate number of paddocks to use, affecting the frequency and intensity of grazing in each system. Assuming an adequate stocking rate, the number of paddocks directly affects the length of the grazing period, the length of the resting period, and the productivity of the alfalfa pasture. All these three aspects may also be influenced by fall dormancy.

The objective of this study was to determine, in a rotational system, the effect of grazing frequency on yield and persistence of alfalfa cultivars belonging to four different fall dormancy (FD) groups. The experiment was conducted at INTAís Marcos Juarez Exp. Stn., Cdba., Argentina during three growing seasons. The treatments were evaluated in a factorial experiment with a split-plot design. Four cultivars, belonging to FD classes 3, 5, 7 and 9, were used as main plots. Four grazing periods of 2, 6, 12 and 18 days were used as subplots. A uniform resting period of 36 days was used for all treatments. As a result, four rotational grazing systems having 19, 7, 4 and 3 paddocks, respectively, were developed. Forage yield was estimated by cutting and weighting 1-m^2 subsamples within each treatment. In the same way, persistence was estimated by counting the number of empty spaces in several 1-m^2 subsamples. It was concluded that: a) Grazing periods for nondormant cultivars (FD 7-9) must be no longer than 7 days; b) The more dormant cultivars (FD 3 to 5) can tolerate longer grazing periods (or fewer number of paddocks) than nondormant cultivars; and c) A resting period of 36 days was appropriate for all tested cultivars.

Comparison of Alfalfa Produced by Hematoxylin Stain and Nutrient Culture Screening

Marihelen Kamp-Glass, David Powell and Guochen Yang. North Carolina A&T State University. 1601 E. Market St., Greensboro, NC 27411

Data for percent germination, percent development of mature plants and biomass data supporting the predictions of the screening processes are presented. Both screening methods yield similar results and match well with biomass data. These procedures, coupled with a multiple shoot organogenesis techniques, are currently being used to develop an aluminum tolerant alfalfa cultivar by breeding only those individuals identified as low pH and aluminum tolerant through seed screening.

Exploiting genome differences for higher alfalfa forage yield

Thomas C. Osborn, Douglas J. Brouwer, and Kimberly K. Kidwell*
Dep. of Agronomy, Univ. of Wisconsin, Madison, WI 53706; *current address: Dep. of Crop and Soil Science, Washington State Univ., Pullman, WA 99164

More heterozygosity in alfalfa cultivars could improve forage yields by increasing the number of loci with favorable dominant alleles and the level of complimentary gene interactions. We previously found that genetic distances of pairs of tetraploid alfalfa genotypes based on molecular markers were significantly correlated with heterozygosity and with forage yields of the single-cross progenies (Kidwell et al. 1994b). Since alfalfa cultivars are synthetic populations developed by intermating many parents, we tested the use of molecular markers for selecting a subset of genetically diverse parents from a population of 93 parents of a commercial cultivar (Kidwell et al. 1998). Based on average pairwise genetic distances between genotypes, four synthetic populations (samples) were developed using 2, 4, 8, 12, 16, or 24 parents selected for genetic dissimilarity (DIS) or similarity (SIM). Forage yields of the synthetic populations varied significantly among samples within parent number and diversity group. However, there was no significant difference between forage yields of DIS and SIM groups averaged over parent number and samples. We concluded that molecular marker selection was generally ineffective due to linkage equilibrium in the population used for selecting parents and the inability to target heterozygosity to specific genome regions affecting yield.

It may be very difficult to extract complementary gene pools that fully exploit heterozygosity from cultivar germplasm because of linkage equilibrium. Two accessions representing the Peruvian and *Medicago falcata* germplasm sources are very unique based on molecular markers (Kidwell et al. 1994a) and could be used to form a novel genetic complement to cultivar germplasm. However, Peruvian is not adapted to northern climates. We obtained some genetic information on adaptive characteristics of Peruvian using two backcross populations from a cross of Peruvian and cv. Blazer XL. These populations were analyzed for molecular markers and a map was constructed of the four homologous cosegregation groups from the F1 for seven of the eight alfalfa linkage groups. The progeny genotypes, replicated by clonal propagation, were evaluated for winterhardiness, fall dormancy and freezing tolerance in two years. For the backcross to Blazer XL, genomic regions affecting all three traits were identified on linkage groups 5 and 8, but regions affecting only fall growth and freezing tolerance were identified on linkage groups 1 and 3, respectively. Multilocus models for winterhardiness in each of two years included 4-5 regions and explained 36-38% of the genetic variation. We are now developing a genetically unique population by intermating Peruvian and *M. falcata* genotypes, and this population will be used to initiate a hybrid selection experiment.

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Molecular Marker Diversity as a Means of Selecting Parents for Synthetic Cultivars

Mary K. Sledge, Joseph H. Bouton, and Gary Kochert
University of Georgia, Athens, GA 30602

Alfalfa synthetic cultivars are produced by intermating large numbers of individuals, often 40 or more. Large numbers of parents are used in order to achieve maximum heterozygosity and avoid inbreeding depression. Yield and heterosis in alfalfa are positively correlated with genetic diversity based on DNA markers. The objective of this study is to use DNA markers to select a small number of genetically diverse parents, to produce high yielding, narrow-based synthetic cultivars. AFLP markers were used to assess the genetic diversity among 120 parents of a ëCUF 101í synthetic cultivar selected for grazing tolerance. Synthetics consisting of 12 and 24 parents were assembled based on genetic dissimilarity (GD) of AFLP markers. The complement of the Dice coefficient was used as a measure of GD, and cluster analysis was used to form groups of 12 and

24 individuals with similar GD scores. From each group, the individual with the highest average number of AFLP bands was selected as a parent for the synthetic. In this way, each synthetic consisted of individuals that were the most genetically diverse, with the maximum number of marker alleles. The 12, 24 and 120 parent synthetics were intermated in the greenhouse to produce seed. Two yield trials and one grazing tolerance trial are being conducted in three locations. The three synthetics were tested for resistance to Anthracnose, *Aphanomyces*, *Phytophthora*, *Verticillium*, and Blue Alfalfa Aphid. In all cases, no significant loss of resistance was seen when narrowing the genetic base from 120 parents to 24 or 12 parents.

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Allele frequency shifts as an indication of marker-trait associations

D. Z. Skinner and D. E. Obert
USDA-ARS and Agronomy Dept. Kansas State University, Manhattan, KS 66506

There are several reasons why co-segregation studies may fail to reveal marker-trait associations in tetraploid alfalfa (*Medicago sativa* L). Several methods for associating markers with traits have been described, each with the stated starting point of a cross of two inbred lines. The development of inbred lines in alfalfa is impossible due to inbreeding depression resulting in virtual sterility after only one or two generations of selfing. The autotetraploid nature of alfalfa vastly complicates the evaluation of cosegregation, due in large part to the diploid nature of the gamete. Reassortment of pairs of chromosomes into the gametes can, under many circumstances, yield apparent recombination between the marker and the gene conditioning expression of the trait (progeny plants expressing the marker or the trait, but not both, from parents expressing both the trait and marker), when in fact, no physical recombination has occurred. Genes occurring in triplex or quadriplex yield no gametes without at least one copy of the gene, hence, only simplex or duplex plants yield segregating progeny. However, gene dosage effects may preclude scoring simplex or duplex plants as "positive" for the trait. Finally, many studies of segregation in alfalfa have shown marked segregation distortion, presumably due to lethal gene combinations.

Instead of relying on segregating populations, it may be possible to use shifts in allele frequencies between populations selected for a trait, and the base, unselected population. Typically, alfalfa cultivars are developed from an initial population largely lacking a particular trait; rare plants expressing the trait are selected and polycrossed, selection is then carried out on that population and the selected plants again are intercrossed. This recurrent selection may be carried out for several generations until the level of expression (both in frequency of plants expressing the trait and the level at which they express the trait) is acceptable. The frequencies of alleles not under selection should be virtually identical in both the base population and the selected population. However, the frequency of alleles that affect expression of the trait must occur with significantly greater frequency in the selected population. Generating large numbers of markers from the selected and unselected populations will detect markers physically associated with genes determining the trait. Unfortunately, the power of statistical tests to discriminate frequency differences diminishes with increasing numbers of markers tested. Resampling analysis techniques can be applied to adjust the significance levels used, and marker-trait associations can thus be defined and tested.

Defining heterotic groups in alfalfa: A molecular marker perspective

E. Charles Brummer
Iowa State University, Ames, IA 50011

Capturing heterosis remains a desirable but elusive goal for alfalfa (*Medicago sativa* L.) breeders. Virtually all commercially marketed alfalfa cultivars are synthetics, developed by successively intermating selected plants and increasing seed through 2-3 generations. The inbreeding present in such a system generally counterbalances any heterosis that may have been expressed in the initial progeny population. Despite repeated efforts along several lines, hybrid cultivars cannot be produced feasibly with current technology. However, the potential for capturing a partial heterotic yield gain has been generally overlooked: some population hybrids have been shown to express high levels of heterosis and could be developed using current seed production processes (Busbice and Rawlings, 1974, Sriwatanapongse and Wilsie, 1968). Population hybrids are actually "semi-hybrids;" half of the progeny will be hybrid (i.e. between populations); the other half will be crosses within each of the parental populations. Due to competition-induced seedling death during establishment, semi-hybrids may yield as much as pure hybrids. In order to express heterosis, two factors are required: dominance at loci controlling the trait of interest, and differences in allele frequencies between the parents at those loci. Most data in maize (Hallauer et al., 1988) and alfalfa (Bingham et al., 1994) show partial to complete dominance to be the genetic basis for yield, and population allele frequency differences have been reported (e.g. Brummer et al., 1991; Kidwell et al., 1994) indicating that alfalfa breeders should be able to realize heterosis. The key to making successful population crosses is to identify or develop heterotic groups and to keep them distinct through breeding, only bringing them together for testing and seed production. The resulting hybrid populations should not be recycled for further breeding to avoid mixing alleles (or linkage blocks) and breaking apart beneficial complementarities between populations. Molecular markers offer several opportunities to capitalize on heterosis: assigning populations to heterotic groups, identifying loci important for the expression of heterosis, selecting parents for multiple or complementary alleles, and identifying particularly useful alleles. Simulations show striking differences in the proportion of multi allelic individuals (i.e. having multiple linkage blocks) resulting from different population matings. Discussions focusing on the inbreeding coefficient, F, cannot easily compare the number of individuals containing multiple complementary linkage blocks (e.g. two-allele and four allele reference populations would each have F=0, even though the former has no individuals with three or four different linkage blocks at a particular locus). Best case crosses are those in which the populations contain contrasting sets of alleles; marker-assisted selection greatly increases the number of individuals with multiple linkage groups. Finally, a method to develop pure population hybrids will be discussed.

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Identifying Patterns of Heterosis in Alfalfa for AFLP Linkage Mapping

Ian M. Ray, Roy G. Cantrell and M. Shaun Townsend
New Mexico State University, Las Cruces, NM 88003

Selection for DNA markers that are linked to loci that control forage yield, quality, and water-use efficiency offers the potential to improve alfalfa production efficiency. The importance of complementary gene action in tetraploid alfalfa and the inability of diploid alfalfa to predict performance of the tetraploid suggest that linkage analysis, and identification of quantitative trait loci (QTL), should be conducted at the tetraploid level. Our goal is to develop a tetraploid alfalfa population that will be useful for both linkage mapping, and for identifying DNA markers associated with QTLs which influence forage yield, quality, and reduced water consumption. The most efficient approach for developing this type of population is to cross genetically diverse parents whose hybrid progeny demonstrate significant heterosis for important traits. Hybrid populations that demonstrate heterosis for such traits should also demonstrate linkage disequilibrium for these traits in subsequent generations. This disequilibrium will be manifested as genetic variation which will be critical for identifying marker-QTL associations. To identify useful parents for linkage mapping, our first objective was to characterize DNA polymorphisms among various alfalfa germplasms that represent much of the genetic diversity in cultivated alfalfa in the U.S. Bulked DNA samples from 30 genotypes of each parental germplasm were scored on an ABI 377 Prism DNA sequencer for amplified restriction fragment length polymorphisms (AFLPs). These data are being used to estimate genetic distances among the germplasms using NTSYS. To identify useful parents for future QTL mapping research, our second objective is to characterize heterotic responses among the germplasms for: forage yield, carbon isotope discrimination, canopy temperature, forage maturity, and forage digestibility. Selected germplasms were crossed in a half-diallel mating design. The hybrid and parental populations have been evaluated in the field for the above traits. Preliminary data indicate significant ($P<0.01$) midparent heterosis for forage yield for several synthetic hybrids. Our third objective is to correlate marker-based genetic distances with field performance of the hybrid populations to determine if molecular genetic distance estimates can predict general and specific combining abilities and heterosis in alfalfa. Based on heterotic response data, and AFLP diversity among the parental germplasms, we will select two germplasms to initiate linkage analysis. A single noninbred genotype from each of the two selected germplasms will be crossed to generate F1 progeny. Our fourth objective is use segregating AFLPs in this F1 population to construct a tetraploid linkage map. To circumvent many of the complexities associated with polyploid linkage analysis we will monitor segregation of AFLPs which behave as single dose polymorphisms (SDPs). An SDP is defined as a fragment that is present in only one parent and segregates 1:1 (presence: absence) in the F1 progeny. Chi square analyses will distinguish between SDPs, which will segregate 1:1, and multiple dose markers which will segregate ≥ 3:1 in the F1 progeny. Linkage relationships of SDP markers will be determined based on two-point and multipoint analysis using JoinMap version 2.0. Ultimately, we will anchor this tetraploid map to a diploid map previously developed by Montana State University and the University of Wisconsin. The AFLP markers used for tetraploid linkage analysis will also be useful in future research to identify QTLs in tetraploid alfalfa.

Potato leafhopper-resistant versus susceptible alfalfa cultivars in New York: A seeding year comparison of yield, quality, and leafhopper feeding damage

J.E. Miller-Garvin, J.L. Hansen, J.K. Waldron, and D.R. Viands
Cornell University, Ithaca, NY 14850

Potato leafhopper (PLH), *Empoasca fabae* (Harris), is the most damaging insect pest of alfalfa, *Medicago sativa* L., in the Northeast. Economically-significant PLH populations may affect seeding year stand survival and lead to reduced forage yield and quality in the following production years. Risk from PLH can be expected annually; however, the severity of infestations is variable from year to year and from county to county. In 1997, seed companies introduced alfalfa cultivars purported to have improved resistance to PLH. Resistant cultivars have the potential to protect alfalfa from PLH injury, reduce insecticide use, and protect forage quality, thereby enhancing net profitability. Our research objectives were to: (1) compare PLH-resistant and PLH-susceptible alfalfa cultivars in the seeding year for yield, PLH damage, and agronomic characteristics, and (2) compare PLH-resistant versus PLH-susceptible cultivars for forage quality and nymphal populations at one harvest/location where PLH damage is severe. Field trials were established in western and central New York (Clarendon and Ithaca, respectively) to increase the probability of encountering damaging levels of PLH. Entries consisted of 19 cultivars, 8 with claims of some level of PLH resistance, 8 susceptibles, and 3 of unknown resistance. Data included yield, PLH damage (1-5 rating scale where 1 = no apparent injury and 5 = severe injury, McCaslin and Miller, 1996), nymphal counts per stem, maturity, and quality. Forage quality data were entered into the FORVAL program (Wilkens and Fick, 1988) and used to calculate dollar value per acre per cultivar. Overall comparisons for yield and PLH damage score were statistically significant at the Ithaca site, which had high PLH populations. Resistant cultivars exhibited significantly higher yields and had lower PLH damage scores. Forage quality and nymph sampling at Ithaca showed that the resistant cultivars had fewer PLH nymphs per stem and had higher percent crude protein and fiber (ADF and NDF), and were more mature. At the Clarendon site, with moderate PLH populations, the resistant cultivars had significantly lower PLH damage scores than the susceptible cultivars, but did not differ significantly in yield. Ranking of the resistant cultivars for PLH damage at both locations was similar. Based on NIRS analysis and FORVAL, the resistant cultivars averaged $31.01 higher hay value per acre in the seeding year than the susceptible cultivars at Ithaca. Although the extreme level of PLH damage observed in the Northeast in 1997 is not expected on a regular basis, the probability that at least one or more of the production years/harvests of an alfalfa stand will be heavily damaged by PLH during the life of the stand is high. In a year with severe PLH pressure, the additional seed cost of resistant cultivars would likely be offset by increased hay yields and quality, and reduced insecticide costs.

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Potato Leafhopper Resistance in Alfalfa: Recent Interpretations

David J. Miller
Pioneer Hi-Bred International, Inc. - Arlington, Wisconsin

Potato leafhopper (PLH) resistant alfalfa varieties were first available to farmers for seeding in 1997. These "first generation" varieties offer a significant yield advantage to growers who do not use insecticide where PLH infestations are significant. However, describing these products for percent resistance and yield across a broad range of locations (PLH infestations) has been difficult.

The reasons for this difficulty include: 1) varying levels of PLH infestations in different years and locations; 2) timing of PLH infestations relative to plant growth; 3) lack of understanding of resistance mechanisms; 4) increasing levels of PLH resistance are raising standards.

In private trials, the advantage of PLH resistant variety 5347LH over susceptible varieties varied from 0.2 to 0.9 t/a in the seeding year, and from 0.1 to 1.6 t/a in the year after seeding. The magnitude of the differences could be attributed to the apparent PLH infestation levels predicted by visual ratings. However, visual ratings did not always reflect yield differences. This situation can occur when PLH infestations by adults occur relatively late in a growth cycle. The result is significant color differences with slight or no yield difference.

Another reason for the discrepancy between visual ratings and yield advantage might be attributed to differences in resistance mechanisms. It appears that some plants and varieties may turn yellow under adult feeding, but have lower nymph counts than susceptible varieties. This may be attributed to differences in a plantís ability to resist adult feeding versus fecundity.

Classification of percent resistant plants for PLH has also been challenging. The standard check variety was released in 1996 as 40% resistant to PLH with an acceptable range of 30-50. After the 1997 season, the values were lowered to 25% resistant with an acceptable range of 15-40. The two major reasons for this change were: 1) more tests were run under severe infestations; and 2) as breeders have improved PLH resistance, the standards are higher for plants to qualify as resistant. In addition to these, other factors that can influence the rating include transplant date, timing of PLH infestation, and cutting schedule.

The development and release of PLH resistant varieties has created many questions regarding varietal performance and the mechanisms of PLH resistance. Testing across a broad range of conditions has helped to answer some of these questions. Further experience with these products, and those with higher resistance will result in better prediction of product performance for growers.

Insights into the Mechanisms of Resistance to the Potato Leafhopper by Glandular-Haired Alfalfa

Floyd W. Shockley and Elaine A. Backus

Department of Entomology, University of Missouri, Columbia, MO 65211

Tube cage tests were conducted on four genotypes supplied by Cal/West Seeds in order to determine the relative resistance and possible physiological mechanisms of that resistance. The differences between these genotypes were defined using a number of parameters: leafhopper mortality, number of excretory droplets (a gross measure of feeding), hopperburn symptoms and severity, stem growth under constant leafhopper pressure, and trichome density and type on various alfalfa plant parts. The G98A genotype performed the best and exhibited the greatest measure of resistance to the potato leafhopper. It caused 100% mortality, low levels of feeding, showed no hopperburn symptoms, and experienced the greatest amount of stem growth under constant feeding pressure. In addition, when trichome density and type were examined, the G98A genotype had the highest ratio of glandular trichomes to non-glandular trichomes on both stems and leaves. In contrast, G98B caused very low leafhopper mortality, moderate feeding, and was relatively susceptible to hopperburn, despite the fact that the number of glandular trichomes vs. non-glandular trichomes were very similar to those found in G98A, the highly resistant genotype. This variability in resistance between morphologically similar genotypes clearly illustrates that the mechanisms of resistance of one genotype may be quite different from those of another. The data from this preliminary study indicate that the nature of the trichome resistance is more than simply a physical barrier preventing the feeding of the insect. In fact, these results strongly suggest that there is a more subtle chemical resistance being utilized by the plant.

Genotype	Mortality (Mean No. ± Std. Dev. of Dead Ins./Cage)	Feeding (Mean No. ± Std. Dev. of Excr. Drops/Cage)	Relative Hopperburn Severity	Stem Growth in cm (Mean No. ± Std. Dev.)		Trichome Density (Mean No. ± Std. Dev./mm) G = Glandular N = Non-Glandular			
				Infested	Non-infested	Stem		Leaf	
						G	N	G	N
G98A	3.00 ± 0.00	404.67 ± 87.54	None	2.67 ± 1.16	6.83 ± 3.69	26.67± 8.62	6.00 ± 3.61	18.33± 5.03	21.00 ±5.57
G98B	0.67 ± 0.58	1040 ± 137.66	Mild	1.50 ± 2.65	7.00 ± 0.50	20.00 ± 4.58	9.00 ± 6.08	8.67 ± 3.51	19.00 ±9.00
G98C	1.67 ± 1.53	974.67 ± 233.61	Severe	1.67 ± 4.62	1.67 ± 1.53	16.00 ± 5.57	5.33 ± 4.16	3.33 ± 2.89	17.67 ± 10.97
G98D	2.00 ± 1.73	1258.33 ± 121.46	Moderate	0.83 ± 0.58	6.50 ± 2.50	14.67 ± 7.37	8.33 ± 6.66	5.00 ± 2.00	23.67 ±7.02

New developments in breeding for resistance to potato leafhopper in alfalfa

Mark McCaslin
Forage Genetics - West Salem, Wisconsin

In 1997 the first potato leafhopper (PLH) resistant alfalfa varieties were commercially released. These varieties have 25-35% resistance to potato leafhopper. Forage yield data from public and private trials, with and without insecticide treatment, show that in unsprayed tests with moderate to high PLH pressure, these resistant varieties offer a 10-20% yield advantage and provide substantial, but not total protection against yield and quality loss due to PLH damage. With insecticide treatment, or very low PLH pressure, the resistant varieties yield 92-98% of the susceptible checks.

The current niche for resistant varieties is areas where PLH damage is common, with growers who do not practice chemical control. Broadening the market niche for these products will require improvements in two areas: increased PLH resistance and improved agronomic performance.

Data on PLH resistance, summarized over three locations in 1997, show the improvement in level of mean resistance for experimental varieties developed in 1994 (GH1), 1995 (GH2) and 1996 (GH3). The table below also compares relative forage yield of these types in a sprayed/unsprayed (Sp/NSp) trial.

PLH resistance and yield (1997-98) in 1997 Sp/NSp test (West Salem, WI)

Variety	% R	ASI	Relative forage yield	
			Spray	Unspray
GH3	62	1.9	97	93
GH2	48	2.6	95	90
GH1	32	3.1	93	89
PLH40	25	3.8	86	81
5454	0	5.0	100	79

Selection for improved agronomic performance has focused on increased recovery after cutting, later fall dormancy and improved persistence. In general, these traits have been negatively correlated with level of PLH resistance. Concurrent breeding progress for increased PLH resistance and improved agronomic performance requires large populations and high selection intensity.

There is a positive correlation to PLH resistance and improved forage quality. Standard forage quality analysis shows a 8-12 point RFV advantage for the glandular-haired types, relative to standard cultivars. In an unpublished animal study by Combs, et. al., palatability (measured as free choice intake), and rate and extent of digestibility were compared between Vernal and the the PLH resistant variety TrailBlazer (no PLH damage on either variety). In this test TrailBlazer showed a statistically significant (p=.05) increase in intake and rate and extent of digestibility. A milk production study was conducted in 1998 comparing TrailBlazer and Blazer XL. Balanced rations were formulated around the differing hay sources and fed to dairy cows in early lactation. In this study, the TrailBlazer ration yielded 4.1 lbs milk/day higher than the Blazer XL check. The basis of the apparent improved forage quality of glandular-haired types is not known.

The challenge for alfalfa breeders will be to incorporate the high levels of resistance required to provide complete protection against PLH losses, improve forage yield potential and maintain the improved forage quality characteristic of the glandular-haired types. Current research suggests that progress is being made on all fronts.

Variation for erect glandular hair density in perennial and annual Medicago

A.W. Lenssen, S.L. Blodgett, S.D. Cash and R.L. Ditterline
Departments of Entomology and Plant, Soil, and Environmental Sciences, Montana State University, MT 59717

We conducted field trials to determine 1) variation on stems for density of erect glandular hairs within and among 8 perennial and 25 annual *Medicago* germplasm, and 2) potential resistance to alfalfa weevil (*Hypera postica* Gyllenhal) provided by erect glandular hairs. Erect glandular hair density and alfalfa weevil damage were determined on about 160 plants of the perennial *Medicago* populations, *M. prostrata*, KS94, KS108, KS159, KS161, KS174, KS210, and KS224, developed by Dr. Edgar Sorensen, USDA, ARS. The perennial eglandular alfalfa entries, 'Arc', 'Ranger', and KS222 were also included. For the perennial entries, erect glandular hair density was determined once in 1995 and four times in 1996. All populations, except for *M. prostrata*, usually were skewed and kurtotic for each sampling date for the density of erect glandular hairs on the first stem internode. Erect glandular hair density ranged from 0-145 mm^{-2}. The perennial population with the highest stem density of erect glandular hairs on stems was *M. prostrata*, which was only sampled in 1995 due to winterkill. Numbers of alfalfa weevil larvae, eggs, and damage were determined three times in 1996. Within perennial populations, correlations between erect glandular hair density and alfalfa weevil eggs, larvae, and/or terminal damage were low and generally nonsignificant. At the population level, mean differences were significant for density of erect glandular hairs at each sampling date, but damage ratings were similar among entries. All Plant Introductions (PIs) with erect glandular hairs from the Annual *Medicago* Core Collection were planted in a replicated field trial and infested with alfalfa weevil adults after appearance of trifoliate leaves. The annual PIs were sampled 11 July 1996 for erect glandular hair density and alfalfa weevil larval infestation. Significant differences for erect glandular hair density were found within and among the five annual *Medicago* species tested, *blancheana*, *disciformis*, *minima*, *rugosa*, and *scutellata*. Among the individual PIs, density of erect glandular hairs on stems ranged from 0-269 mm^{-2}. However, none of the annual entries were infested by alfalfa weevils. Under the conditions of these trials, density of erect glandular hairs differed significantly within and among annual and perennial alfalfa populations, but erect glandular hairs did not confer resistance to the alfalfa weevil in the perennial *Medicago* lines.

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Potato Leafhopper Resistant Varieties in Minnesota

C.C. Sheaffer, N.P. Martin, W.D. Hutchinson, and B.D. Potter

University of Minnesota, St. Paul and Lamberton, MN

The potato leafhopper (PLH), *Empoasca fabae* (Harris), is the most serious insect pest of alfalfa in Minnesota. The insect causes significant economic losses to alfalfa every other year. We evaluated the yield and quality of recently marketed glandular haired, PLH resistant varieties in the seeding year at two locations in southern Minnesota during 1997. Four PLH resistant varieties (5347 LH, Ameriguard 301, Arrest, and DK 121 HG and two check varieties (5454 and Vernal) were grown with and without insecticide (Permethrin) application. Alfalfa was seeded in early May and harvested in July and September in the seeding year. Potato leafhopper populations above economic thresholds occurred at all locations before the July harvest with peak populations in untreated areas of 6 and 21 PLH per sweep at Rosemount and Lamberton. Leafhopper populations were less during the regrowth before the September harvest but exceeded economic thresholds when peaking at 1.2 and 1.6 per sweep at Rosemount and Lamberton.

Potato leafhoppers caused yellowing of alfalfa and reduced alfalfa height and yield at both locations although effects on yield were less at Lamberton than at Rosemount. Resistant varieties had less injury and greater first cut and total yield than susceptible varieties when no insecticide was applied; with insecticide application, resistant and check entries had similar yields. Resistant varieties differed in symptoms resulting from PLH feeding. Leafhoppers reduced forage crude protein concentration but increased relative feed value (RFV) when no insecticide was applied. Enhanced RFV was associated with decreased height, decreased stem mass and increased leafiness. Resistant varieties had greater RFV than check varieties. Resistant varieties provided greater net returns (based on RFV production per acre) than check varieties and returns for the resistant entries were greater when no insecticide was applied than when insecticide was applied. Our results indicate that seeding year alfalfa may be more sensitive to PLH than established stands and that use of PLH resistant varieties increases the profitability of alfalfa production.

New Economic Thresholds for Potato Leafhopper-Resistant alfalfa

Stephen A. Lefko, Larry P. Pedigo, and Marlin E. Rice
Iowa State University, Department of Entomology

An economic threshold (ET) is the pest density at which management action should be taken to prevent an increasing pest population from reaching the economic injury level (EIL). The EIL is the lowest number of insect pests that will cause economic loss equal to the cost of management. The severity of an insect pest can be explained by the distance between its general equilibrium position (GEP) and its economic threshold for a given crop (Fig. 1). A greater distance between the two will lower the frequency that the population number exceeds the ET. Virtually all but one pest management tactic widens the gap between the GEP and the ET by suppressing the pest number viz., tolerance (Fig 1.). Tolerance is one of the three host plant resistance mechanisms that raises the threshold instead of lowering the pest density and increases the longevity of the management tactic. Several studies have shown the undesirable consequences of pest suppression, some of which are: pest replacement, pest resurgence, and selection for resistant strains. Recent studies have provided strong evidence that Leafhopper-resistant alfalfa has a large degree of tolerance.

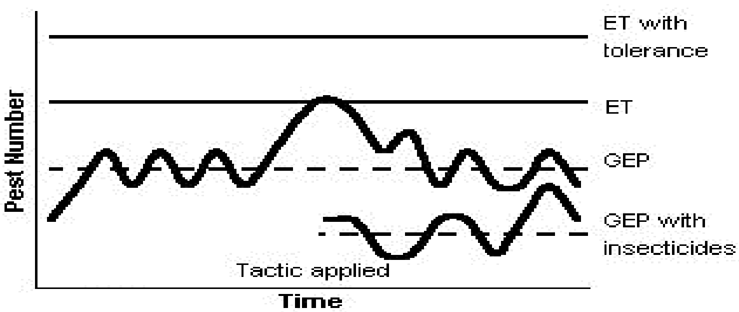


Figure 1. Stylized graph of the relationship between the GEP and ET for tolerance and insecticide tactics of pest management.

Different densities of adult potato leafhopper were caged in the field on the same five alfalfa varieties. These trials produced data on yield response to increased leafhopper pressure and leafhopper population growth on small populations alfalfa. The economic threshold for the susceptible variety during the first-year second-cutting was approximately 0.1 adult leafhopper per sweep per inch of alfalfa. Using the same valuation criteria, the economic threshold for resistant alfalfa was 0.7 leafhoppers per sweep per inch of alfalfa. Moreover, there is evidence this threshold increases with the age of the stand. Although there was a difference in the yield response, there was no difference in the number leafhopper nymphs collected from resistant and susceptible cages. Piecing these results together, it appears first generation leafhopper-resistant alfalfa can increase the economic threshold without effecting the GEP. This is an optimal tactic and likely will become the cornerstone of integrated pest management in alfalfa regularly plagued by potato leafhopper.

Observations on Glandular Haired Alfalfa in Wisconsin:

Performance and Mechanisms of Resistance.

David B. Hogg, Daniel J. Undersander, John L. Wedberg and Kevin G. Silveira

Departments of Entomology and Agronomy, University of Wisconsin, Madison, WI 53706

The potato leafhopper (PLH), *Empoasca fabae* (Harris) (Homoptera: Cicadellidae), is considered the most significant insect pest of alfalfa in Wisconsin and throughout the upper Midwest. Until recently, effective management of this pest could be achieved only by scouting and applying insecticide when warranted. However, the commercial development of glandular haired alfalfa varieties holds promise for managing PLH in alfalfa with little or no insecticide inputs.

In anticipation of the 1997 release of glandular haired varieties, a four state trial (Ohio, Indiana, Minnesota and Wisconsin) was established in 1996 to evaluate the field performance of the new varieties. Five standard ("susceptible") commercial varieties and nine glandular haired ("resistant") entries were included in the trial. Results for the seeding (1996) and first production (1997) years varied by location. In Wisconsin, PLH infestations were light (1996) to moderate (1997), and in general the resistant varieties showed no yield advantage over the susceptible varieties, although in both years sprayed plots (PLH controlled with insecticide) produced significantly higher yields than unsprayed plots for both resistant and susceptible varieties. In contrast, at sites experiencing heavy PLH infestations (Ohio and Indiana), in the absence of insecticide sprays yields of the resistant varieties were significantly higher than yields of the susceptible varieties, although again sprayed plots produced significantly higher yields than unsprayed plots, for the resistant as well as the susceptible varieties

These results support a general perception that the benefits of glandular haired varieties may not be apparent under light to moderate PLH pressure but are measurable when pressure is more extreme, and that currently plant resistance alone does not eliminate damage and yield loss. This begs the question of the mechanism(s) of resistance in glandular haired alfalfa. But rather than simply classifying resistance as antibiosis, nonpreference or tolerance (information from various sources suggests that all three may be involved), it might prove instructive to examine the alfalfa-leafhopper interaction at a more fundamental level, specifically recognizing that it involves two interacting populations, the alfalfa plant population and the insect pest population. To this end we offer the following comments. An obvious issue from the plant perspective is the genetic and phenotypic variability among the plants in a population (i.e. variety). Clearly, the percentage of resistant plants is important, but perhaps of equal importance is variability in the degree of resistance among those "resistant" plants. From the insect perspective, an issue of overriding importance is that PLH adults are known to utilize scores of different plant species as hosts and thus have many choices in addition to alfalfa of where to feed and lay eggs. Also, PLH adults are not very discriminating about which plant species they colonize, and it is emigration (the "leaving rate") rather than immigration that determines where the insects stay and populations develop. Therefore, how PLH adults perceive the glandular haired plants they encounter in a field planted to a resistant variety may be an important determinant of how many remain to feed, reproduce, and ultimately damage the alfalfa.

Physiological Assessments of Alfalfa Autotoxicity.

C. Jerry Nelson, Sang-Uk Chon and John H. Coutts
Agronomy Department, University of Missouri, Columbia, MO 65211

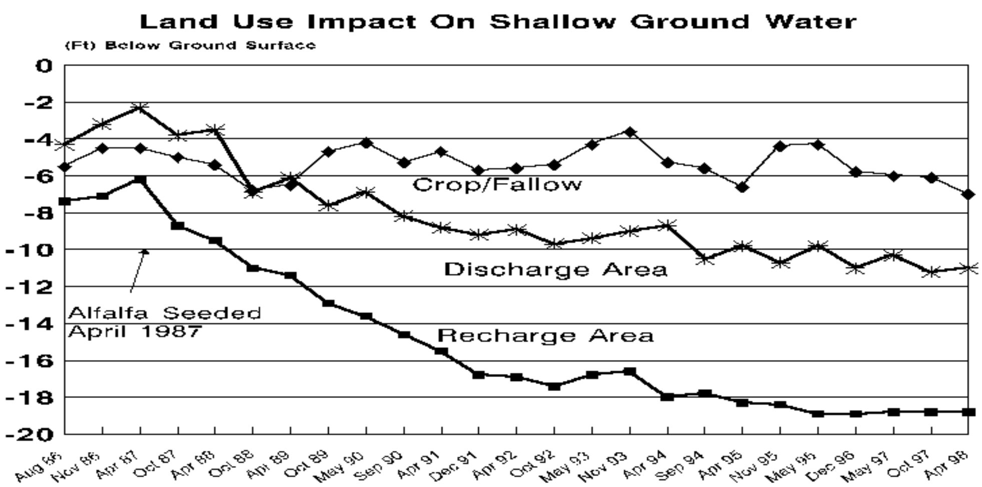
Autotoxicity restricts reseeding of alfalfa after alfalfa until the autotoxic chemical, or chemicals, breaks down or is dispersed, often requiring up to a year or more. The usual response to the autotoxin is reduced root growth, but at high concentrations it also delays and reduces germination. A solution could be to select cultivars with tolerance to the allelochemical(s). Thus, we began working on bioassay procedures of seed germination and early seedling growth for germplasm evaluations. We use water-soluble extracts of field-grown alfalfa herbage and an agar-based petri-dish assay in light. We found more than 2-fold genetic variation for tolerance among cultivars and breeding populations at low concentrations of extract. Here we report on relative effects of the autotoxic chemical on the sequential rates of seed imbibition, seed germination (when radicle is emerged 1 mm) and early seedling growth, and compare the physiological responses. Seed imbibition until a stable water content is reached is affected very little at extract concentrations (0.5-2.0 g dry tissue per liter of water) that mainly reduce root elongation. Imbibition is slowed slightly at higher concentrations (more than 8 g per liter) that also reduce germination percentage, but imbibition is likely not a major factor in most situations. Therefore, we routinely imbibe seeds in water for 12 hours to obtain more uniform germination. We measured genetic differences in several subsequent processes among 18 or 20 germplasms and cultivars in three separate experiments. Path coefficient analysis showed germplasms with larger seed size (mg per 100 seed) are slightly slower to reach 50% of final germination, but root growth rates (mm per day) or radicle and hypocotyl lengths at 5 days are not affected by seed size in control or extract solutions. This suggests that large seed size is not a factor that confers tolerance to the autotoxin. Radicle length at 5 days after imbibition is more sensitive to the allelochemical than is hypocotyl length. Path coefficient analysis showed genetic variation in root length is 9 to 17 times more important than variation in hypocotyl length for determining total seedling length in control or extract treatments, again pointing out the significance of the radicle in the autotoxic response. Genetic variation in root growth rate and radicle length at 5 days are greatest and easiest to detect with an extract concentration of 1 g dry tissue per liter (0.1%), a concentration that delays germination slightly, has no effect on final germination percentage, but reduces root growth of the average germplasm to about 50 to 60% of the control. Among the 18 or 20 germplasms and cultivars radicle length in a 0.1% extract ranged from about 30% to near 100% of control. Thus far we have evaluated genetic tolerance as percent of control because genetic variation exists for radicle length in control treatments, but the variation on a relative basis is much less than that in the 0.1% extract treatment. In fact, genetic differences in radicle length at 5 days in control treatments are not related to tolerance, but lengths in 0.1% extract are highly correlated (0.61, 0.84, and 0.95) with measurements when data in 0.1% extract are expressed as percent of control. The correlations between length in 0.4% extract and the percent of control were 0.65, 0.92, and 0.94, but the germplasm means and variances were lower than at 0.1%. In summary, to screen for genetic tolerance, radicle length at one concentration of extract could be used as a bioassay, considering that seeds need to germinate at the same time to give equal seedling ages when measured. The toxicity level should reduce radicle length of the average germplasm by about 50%. A control treatment would be needed to determine the relative toxicity level of the soil or bioassay environment.

Solving Saline Seeps Using Perennial Forage Rotations

Jane M. Holzer, Scott K. Brown, Robert G. Legare and Joseph J. Von Stein
Montana Salinity Control Association, Conrad, MT 59425

Salinization of soil and water resources occurrs on several million acres in the Northern Great Plains, with over 300,000 acres of Montana cropland affected. The nonpoint source pollution degradation is primarily caused by the large scale adoption of the crop-fallow dryland farming system since 1940 which replaced native rangeland conditions. Inefficient use of precipitation allows deep percolation of soil moisture and migration of soluble salts. The build-up of shallow saline ground water combined with certain geologic conditions leads to the development of low volume saline springs called saline seeps. Irrigation practices and water delivery systems can also be implicated in saline seep development.

The most successful control practice is growing deep-rooting high water-use perennial crops in critical up-slope recharge areas. A short-term rotation of alfalfa alone or in a forage mixture will lower the overall ground water level gradually, and thus allow rainfall to leach the accumulated salts back down into the profile. The ratio of recharge area to saline discharge area averages 10:1, varying with geologic conditions. The perennial forage rotation can be as short as five years if 80-100% of the recharge area is addressed and up to 20 years if less acreage is planted. Montana Salinity Control Association is a conservation district based multidisciplinary technical field team to provide recharge area identification and site-specific reclamation plans.



Saline discharge area plantings alone are not effective and at best only slow the growth; however, the reclamation process can be enhanced if combined with recharge area plantings. Alfalfa is not tolerant to the saline soil and high water table conditions; therefore, any discharge seedings are salt-tolerant grass species with alfalfa as a small component.

Watershed management strategies are required when salinized conditions expand beyond individual saline seeps scattered throughout cropland to being a predominant image across the landscape damaging waterways, streams, reservoirs and land. Resource protection then requires widespread adoption of intensive annual cropping practices and utilizing deeper rooted perennial forage crops. Annual crop production must expand beyond traditional cereal grains to encompass pulse and oilseed crops to avoid pest problems. The USDA Federal Farm Bill has several incentive programs to encourage perennial forage and improved water management. Two widely used programs in Montana are Conservation Reserve Program(CRP)and Environmental Quality Incentive Program (EQIP).

VARIABLE SEEDING RATES FOR ALFALFA: YIELD AND FORAGE QUALITY

A. Gray, L. Bjornestad, J. Despain, J. Gill, J. Jenkins, J. Langbehn, A. Mesbah, and M. Ryan.
Powell Research and Extension Center, Powell, WY 82435

High seeding rates increase alfalfa establishment cost, but are sometimes proposed to increase alfalfa hay quality. Proponents maintain that plant competition in dense stands tends to decrease stem diameter and increase forage quality by decreasing fiber content. A project was established at Powell, Wyoming to determine if variable seeding rates influence yield and forage quality of alfalfa. This project was established on an irrigated, clay loam site at the PREC on April 30, 1997. Plots (15 ft by 30 inches) were planted on a mellow seedbed (furrow-corrugated in 36 inch beds) with a Carter five-row planter (five-inch row spacings) on April 30, 1997. Two hundred thirty pounds of 11-52-0 were soil-incorporated prior to plot establishment. Eleven seeding rates of Fortress alfalfa (from 6 to 26 lbs/A PLS in two-pound increments) were established in a randomized-block design with four replications. All plots were treated with a post-emergent herbicide combination (2,4D-B @ three quarts/A and Poast Plus @ 60 oz/A) on June 4. The trial was gravity-flow irrigated five times (24 hour sets) from early May to early

September. Forage plots were cut with a REM flail harvester. Two harvests were taken in a stage of 50% bloom. Forage sub-samples from each plot were analyzed for both crude protein and relative feed value. In 1997, yield and forage quality were not significantly different among seeding rates for the first harvest (July 23). Second harvest (October 1) and season total yields for 24 and 26 lb seeding rates were significantly lower than yields for some of the lower seeding rates. Crude protein and relative feed values were not significantly different among seeding rates for the second harvest. Higher seeding rates did not significantly increase forage quality for any harvest during the establishment year. Even when the highest two seeding rates appeared to diminish yield on second harvest, quality did not appear to be influenced during the establishment year. In 1998, first harvest yields were taken in late bud stage on June 11. Yield for the 8 lb/A seeding rate (3.38 T/A) was greater (P=.05) than the yield (2.67 T/A) for the 26 lb/A seeding rate. Season-long yields will be evaluated again for the 1998 season. Forage qualities among all seeding rates will be compared for first-cutting yields. Stand density of the 6 lb/A seeding rate (10.5 crowns/sq.ft) was different (P= .05) from the 26 lb/A (16.9 crowns/sq.ft.) seeding rate on April 9, 1998. Stand densities will be evaluated again in 1998.

Alfalfa Information for Producers Via WWW

John Caddel, Jim Stritzke, and Richard Berberet

Oklahoma State University, Stillwater, OK 74078

The Oklahoma Alfalfa Production Calendar on the World Wide Web is designed to supply practical and timely alfalfa production information. Its URL is http://www.agr.okstate.edu/alfalfa/pageone/alfa-cal.htm

Contents and Purpose: The Oklahoma Alfalfa Production Calendar contains applied information to assist in profitable production of high quality forage desired for marketing and use in Oklahoma and the surrounding area of the Southern Great Plains. Information supplied has long-term applications such as those relating to stand longevity and immediate utility such as updates on current insect infestations. Cost-benefit relationships are addressed in most topics.

Audience: The ultimate users of the Production Calendar are alfalfa producers; however, much of the information may reach them through County Extension Educators, Agricultural Consultants, and Crop Scouts who use the site. Lending agencies and material suppliers should also benefit from this web site.

Organization: The Calendar centers on the month and production categories. Users select the month of interest, then indicate the type of production, storage, or marketing information desired. Most months include the following choices: Equipment, Forage Quality, General Production, Gopher Control, Grazing, Harvest Management, Hay Storage, Insect Control, Liming Acid Soils, Machinery, Marketing, Seed Production, Soil Fertility, Soil Sampling,

Stand Establishment, Tools, Variety Choices, Water, Weather Links, and Weed Control. Information is arranged from short statements to more in depth discussions of the topic and to references of publications with additional information. Included are "bullet statements" (1 or 2 sentences) that serve as reminders to experienced alfalfa producers, "production tips" (150 to 500 words) that provide new information about products, processes, and research), "information capsules" (1 to 10 sentences about a particular alfalfa topic, including trivia), OSU Extension Facts (2 to 8 pages), OSU Extension Circulars (10 to 30 pages), and other references. Several hundred photos are provided to help understand the problems and opportunities for alfalfa production.

Developers: All alfalfa research and extension personnel at Oklahoma State University contributed in various ways to the Oklahoma Alfalfa Production Calendar. Most of the text and web page design was developed by John Caddel (Forage Agronomist, Plant and Soil Sciences Department), Jim Stritzke (Weed Specialists, Plant and Soil Sciences Department), and Richard Berberet (Alfalfa Entomologist, Entomology and Plant Pathology Department). Critical reviews were received from other members of the Oklahoma Integrated Management Team. Chad George, Jason Warren, and Kerry Stromski, undergraduate students, helped to create formats and verify links.

Sponsors: Funding for the Oklahoma Alfalfa Production Calendar was provided by the Oklahoma Agricultural Experiment Station, Oklahoma Cooperative Extension Service, USDA/CSREES Southern Regional IPM Grant No. 97-EPMP-1-0146 and, and the Oklahoma Alfalfa Hay and Seed Association. Server space and administration was provided by the Plant and Soil Sciences Department, Division of Agricultural Sciences and Natural Resources, Oklahoma State University.

Future: New information will be incorporated as it becomes available. Links to other web pages will be included, and as the audience contributes questions and comments, new text and photographs will be included. Comments to assist in the development of the Web Site are welcome and can be sent to jlc@soilwater.agr.okstate.edu

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Alfalfa for Electricity Generation: Variety and Harvest Schedule Evaluation

C.C. Sheaffer, N. P. Martin, J.F.S. Lamb1, and G. J. Cuomo
University of Minnesota and USDA-ARS1, St. Paul, MN 55108

The strategy for use of alfalfa as a biofuel is to separate whole plant hay into leaf and stem fractions with stems being gasified and leaves processed into leaf meal for livestock feeding. Optimal harvest schedules for production of alfalfa for electrical generation are unknown. Typically, alfalfa in the Midwest is harvested three or four times per year. A less frequent, two-cut system may be most effective for stem and leaf co-product production, allow harvest flexibility, and provide wildlife habitat. We evaluated the impact of two-, three-, and four-cut harvest regimes on leaf and stem yield and composition of alfalfa germplasms and varieties at three locations for two years.

Two harvests per season at late flower resulted in greater stem yields than three harvests per season at early flower. Leaf yields were consistently lower for the late flower harvest than for the early flower harvest. Harvest regime did not affect total yields at three locations-years, but at the other three location-years, yields were greater for the early flower harvest regime than for the late flower harvest regime. Leaf and stem quality decreased with increasing maturity at harvest. For example, at Rosemount, leaf CP concentration for the mid-bud, early flower, and late flower harvest regimes was 31, 30 and 28%, while stem CP concentration was 14%, 11%, and 10%, respectively. Harvest regime also affected macro- and micro-mineral concentration of stems with concentrations declining as maturity at harvest decreased. There were few harvest regime by entry interactions. Entries seldom differed in leaf or stem yield, but WL 252 HQ, MP 2000 and ABI 9239 often had greater leaf CP concentration, greater stem CP, lower stem ADF and greater leaf concentration than other entries. Entries did not differ in stem mineral concentration.

An economic analysis was conducted that valued stems as a fuel and the leaf co-product as a livestock feed. Although the two-harvest regime was less costly than a three-harvest regime, greater leaf yield and quality for the three-harvest regime resulted in greater net return per acre.

Reference

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Pacific Rim Forage Exports

W. P. Ford
Cooperative Extension, Washington State University
Pasco, Washington, 99301, U.S.A.

Exports are an important market for the Western United States forage producer. Forage products exported are: alfalfa hay and cubes, timothy hay, oat hay, sudangrass hay, bermuda hay, and perennial ryegrass and fescue straw.

During 1997, the United States exported 1,960,636 metric tons (mt) to the Pacific Rim. Japan was the largest market at 89.6% or 1,757,230 mt. Korea was the second largest destination at 5.7% or 111,214 mt. Taiwan was the third largest destination and received 4.5% or 88,454 mt. The countries of China, Indonesia, and the Philippines only received 0.2% or 3,738 mt. The Port of Seattle was the largest port at 490,112 mt of forage products exported to the Pacific Rim.

In 1997, Japan imported 650 thousand metric tons (mt) of alfalfa cubes, 1.757 million mt (mmt) of baled hay, and 266 thousand mt of alfalfa pellets. The United States supplied approximately 74% of these cubes, 83% of the baled hay, but less than 2% of the pellets. Overall, the United States supplied about 73% of Japanís total 1997 forage imports (hay, cubes, pellets) of 2.674 mmt.

The United States Department of Commerce data indicate that the PNW share of the 1.96 mmt exported is about 53% or 1,037,802 mt. Forage exports represented about 9.7% of total hay production in Washington, Oregon, and Idaho in 1997. In 1996, approximately 535,000 mt of forages were exported from Washington. Exports from Washington state in 1996 represented 18.8% of its total hay production. This is the third year that forage exports from Washington have exceeded $100 million. Forage exports the past ten years have helped support and stabilize forage prices in the PNW even with increasing acreages and variable market conditions.

The United States and Canada will continue to be major suppliers of forage products to Japan. Baled hay exports have dramatically increased during the past 9 years, with the United States supplying the vast majority of products. Alfalfa cube exports from the United States have declined somewhat and Canada has increased its market share the past few years.

The Korean market is starting to develop and is now the second largest destination for forage products. Korea displaced Taiwan because of population, livestock numbers, limited arable land base, and a larger economy. In 1997, Korea imported 56,526 mt of alfalfa cubes; 34,451 mt of alfalfa hay; 77,450 mt of grass straw; and 53,396 mt of alfalfa pellets. The United States supplied approximately 53% of these cubes, 87% of the baled alfalfa hay, 69% of the grass straw, and zero percent of the pellets. Overall, the United States supplied 51% of Koreaís total 1997 forage imports of 221,823 mt. The potential size of this market is estimated to be one-quarter to one-third of the Japanese market or 500,000 to 600,000 mt of hay and cubes. The forage market in Taiwan is now the third largest Pacific Rim consumer for the United States. Total exports to Taiwan have leveled off the past two years. It is not clear whether these import levels will continue or drop to lower levels.

Washington and the West Coast forage industry need to recognize the importance of Pacific Rim exports to the forage economy of their areas. The PNW needs to export quality hay, cubes, and straw; promote consistency of product; and cheaper transportation methods to retain and/or increase their market share in a growing world market.

Value of the Grazing Tolerant Trait for Nondormant Alfalfa Cultivars Used as Supplemental Grazing by Beef Cows and Calves

Joe Bouton[1], Roger Gates[2], Jerry Baker[3], and Garry Hill[3]
[1]Dept. of Crop and Soil Sciences, University of Georgia, Athens, GA 30602; [2]USDA-ARS and [3]Dept. of Animal Sciences, Coastal Plain Experiment Station, Tifton, GA 31793

Perennial, warm season grasses are the predominate pasture species in the coastal plain region of the southern USA. These grasses have two major limitations: 1) an off-season (normally winter) when they have limited or no production and 2) poor nutritional value during most of their main growing season. Using nondormant alfalfa as supplemental grazing for grass pastures has potential to overcome these limitations because it possesses a long growing season in areas with mild winters and also has high nutritional quality. However, alfalfa is generally not grazed due to poor stand persistence and grazing tolerant cultivars are currently dormant types. Therefore, selection after intense grazing was used to develop two nondormant, grazing tolerant alfalfa cultivars, AmeriGraze 702 and ABT 805 (Bouton et al. 1997a; Bouton et al. 1997b).

In this current study, we are evaluating the value of the grazing tolerant, nondormant alfalfa cultivars when used as supplemental pasture in a grass-based beef production system. Twenty British breed cows, their calves and one bull during the breeding season, have access to either 20 acres of Tifton 85 bermudagrass (*Cynodon dactylon* L.) pasture alone or Tifton 85 bermudagrass plus 2 acres of alfalfa. Large, replicated strips of four cultivars comprised the alfalfa area. These cultivars include AmeriGraze 702 and ABT 805 plus the checks Alfagraze (dormant, grazing tolerant) and Florida 77 (adapted but grazing intolerant nondormant). In each of two years, cows and calves were limit grazed from approximately April 1 to June 15, followed by creep access for calves until weaning. Limit grazing involves morning access to alfalfa for several hours 3-5 days per week, depending on forage availability. The alfalfa field is split in half with electric fence across the cultivar strips. Rotational access helps maintain leaf area at levels sufficient to stimulate alfalfa growth. Rotation continues during summer while calves have continuous access to alfalfa through a creep gate. If alfalfa accumulates, hay cutting or mob grazing with cows can remove excess forage, preventing excessively mature forage. Calves return to bermudagrass pasture and have continuous access to alfalfa following weaning.

After two seasons grazing, Alfagraze, AmeriGraze 702, and ABT 805 showed better stand survival and less weed encroachment when compared to Florida 77. Fall and early spring growth was observed to be better on all the nondormant cultivars than Alfagraze. Generally, a numerical, but non-significant, increase in milk production was found for cows with access to alfalfa. No difference in calf weaning weight was measured between the two systems. Post-weaning performance of calves with access to alfalfa was comparable to contemporaries fed a conventional hay and grain weaning ration. For calves with access to alfalfa post-weaning, performance of animals with prior exposure to grazing alfalfa was greater than those without prior experience.

This grazing system with alfalfa can provide a cheap, dependable source of supplementation for southern cow/calf producers. The value of nondormant cultivars with the grazing tolerant trait, even using this form of rotational grazing, was evident by increased stand survival, better fall growth, and less weed encroachment.

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Biologic and Economic Assessment of Herbicide Use in Alfalfa in the U.S.

R. Gordon Harvey, Arthur A. Hower and Jayson Harper
Dep. of Agronomy, Univ. of Wisconsin-Madison, Madison, WI 53706

A survey sponsored by the National Agricultural Pesticide Impact Assessment Program identified 94 weeds (individual species or groups of similar species) of economic importance in alfalfa grown in the U.S.. Although ranking of weed importance varied by region and alfalfa seeding or production method, the highest ranked summer annuals were foxtail species, pigweed species and common lambsquarters, winter annuals were field pennycress, shepherd's-purse and downy brome, and perennials were quackgrass, dandelions and curly dock. Musk thistle was the only biennial weed identified as a problem in alfalfa. Dodder and prickly lettuce were identified as consistently important weeds in alfalfa grown for seed.

The survey reported an average of 23 million acres of alfalfa grown for hay and 179,000 acres of alfalfa grown for seed from 1988 to 1992 in the U.S.. During that period, an average of only 16.6% of the alfalfa hay acreage was treated with herbicides, whereas an average of 78.3% of the alfalfa seed acreage was treated with herbicides. The percentage of alfalfa hay acreage treated with herbicides varied greatly between regions. Although nearly 74% of the U.S. alfalfa hay acreage was located in the North Central region, only an average of 8.1% of that acreage was treated with herbicides. In contrast, 50% of the alfalfa hay acreage in the Western region was treated with herbicides.

Approximately 3.2 million acres of alfalfa were spring seeded annually between 1988 and 1992 for forage production. Thirteen herbicides were used for weed management in spring-seeded alfalfa (Table W6). Glyphosate, imazethapyr and 2,4-DB were used on the greatest acreage in the North Central region. In the North East region, 2,4-DB, EPTC, imazethapyr and glyphosate used on the greatest acreage, while EPTC, 2,4-DB and bromoxynil were used on the greatest acreage in the West. Glyphosate was used on the greatest acreage for the U.S. as a whole. Approximately 1.4 million acres of alfalfa for forage were fall seeded annually between 1988 and 1992. Twelve herbicides were used on fall-seeded alfalfa for hay. Paraquat and 2,4-DB were applied to the most acres overall, but EPTC was used in greatest quantity. Paraquat was used on the highest percentage of the fall-seeded alfalfa forage acreage in the North Central region, whereas imazethapyr was used on the highest percentage of fall-seeded acreage in the North East region, and 2,4-DB and EPTC were used on the highest percentage of fall-seeded acreage in the West. Twelve herbicides were used for weed management in established alfalfa grown for forage. Metribuzin, sethoxydim and paraquat were the most extensively used herbicides in the North Central region. Paraquat, hexazinone, terbacil, metribuzin and imazethapyr were the most extensively used herbicides in the North East region. Metribuzin and hexazinone were the most extensively used herbicides in the West.

Use of 12 herbicides was reported in both spring and fall seedings of alfalfa grown for seed. Benefin, 2,4-DB, bromoxynil, sethoxydim and EPTC were used on the greatest acreage of spring seedings, while 2,4-DB, benefin, bromoxynil, sethoxydim and EPTC were used on the greatest acreage of fall seedings. Seventeen herbicides were used in established stands of alfalfa grown for seed, with diuron, hexazinone, EPTC, trifluralin and ethalfluralin being used on the greatest acreage.

Early Spring Grazing of Alfalfa for Efficient Forage Utilization and Pest Management

T.C. Hale, R.C. Berberet, and J.F. Stritzke Oklahoma State University, Stillwater, OK 74078

The primary goal of this research is to increase profitability and sustainability of alfalfa forage production by using early spring grazing or early first harvest along with carefully-timed pesticide applications to manage insect pests and cool season weeds while promoting more efficient harvest scheduling. Pest species targeted with these treatments include the alfalfa weevil (AW), *Hypera postica* (Gyllenhal); blue alfalfa aphid (BAA), *Acyrthosiphon kondoi* Shinji; cheat, *Bromus secalinum* L.; downy brome, *B. tectorum* L.; and shepherdspurse, *Capsella bursa pastoris* L. Harvest treatments used in this study also serve to adjust the timing of first harvest away from the traditional late April-early May schedule when the probability of rainfall is at its highest annually.

This research is being conducted in Grady County (Southcentral Oklahoma Research Station) using 'Garst 630' alfalfa during the 3rd through 6th years of production. A split-plot design is being used with 7 replications of harvest treatments on main plots (9 X 10 m) and pesticide treatments on subplots (5 X 9m). Harvest treatments consist of l)grazing for 3-4 weeks during March /April; 2)prebud cut at peak prevalence of alfalfa weevil larvae (mid-April); and 3)early bloom cut according to the typical schedule (late April/early May). Subsequent harvests are taken at ca. 35-day intervals with some adjustments in timing as needed to bring all into synchrony by late summer and provide a consistent overwintering habitat for insects. Treatments on subplots are pesticide applications as needed to control insect pests(carbofuran and permethrin) and weeds (hexazinone, sethoxydim, and imazethapyr) vs. no-pesticides. Infestation levels for weeds are determined during February (cool-season) and by estimating the percentage of forage comprised by weeds at each harvest. Population densities for AW larvae and aphids are determined at ca. weekly intervals during growth of the first crop of alfalfa by pulling samples of 25 stems from each subplot and extracting of insects from foliage in Berlese funnels for counting. The amount of alfalfa consumed in grazing is determined by weighing dryed herbage from quadrates (0.5m2) cut from ungrazed areas at the time cattle were removed. A flail-type harvester is used for forage samples (5m2) from each subplot for yield estimates and stand densities (stems/0.1m2) are estimated for each crop.

Infestations of BAA,and AW during March/April of each year have been effectively reduced by grazing so that the need for insecticide was limited over the 3 years to a single application of Pounce in 1997. Weed interference has also been greatly reduced by grazing to the extent that weed content of forage with and without herbicide has been much lower than in the prebud and early bloom treatments for the duration of this research. Due to a devastating infestation of BAA in 1997, no alfalfa was cut at prebud and a mean of just 312 lb/acre at early bloom first harvest without insecticide. Seasonal yields in 1997 for these treatment combinations were significantly lower than for all other treatments. After 3 years, there were no significant differences in stand density among harvest treatments when pesticides were used as needed. Stands in treatments without pesticides were significantly reduced in comparison to those with pesticides in all harvest treatments. Overall, spring-grazing did not result in significant differences in yields or stand densities in comparison to the prebud or early bloom harvest schedules.